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A computer program for analysis, simulation and optimization of asymmetric catalytic processes proceeding through two consecutive steps. Type 2: sequential kinetic resolutions

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Abstract: A computer program was developed for the treatment of sequential kinetic resolutions, i.e. a kinetic resolution of a bifunctional racemate which proceeds via two consecutive steps. The program allows the analysis, simulation and optimization of such processes and it facilitates the determination of the point of the reaction at which each species — either the remaining substrate, the reaction intermediate or the final product — can be harvested with a maximum in chemical and/or optical yield. A case study shows that, provided the selectivities of the individual steps are matching, sequential resolutions offer considerably higher enantiomeric purities of product(s) as compared to conventional single-step processes. In an extension to previous studies, which recommended that the rate of both steps should be equal to achieve an optimal reinforcement of the selectivities, it was shown that systems which do not fit into this pattern can also lead to products having a maximum in chemical and/or optical yield as long as the right species — either the remaining substrate, the reaction intermediate and/or the final product — is harvested at the appropriate degree of conversion. © 1997 Elsevier Science Ltd

Introduction

Despite some inherent disadvantages — the most striking being a 50% theoretical yield of each enantiomer — kinetic resolution of racemates is one of the major sources for enantiomerically pure compounds.[1,2] The other sources are asymmetrization reactions[3,4] and compounds from the 'chiral pool'. On a closer look, however, the latter offers far less material than generally assumed, when the price is considered.[5] As a valuable alternative, biocatalysts have proven to be extremely flexible for the kinetic resolution of man-made organic compounds over the last decade.[6]

To ensure high selectivity of a single-step kinetic resolution — most conveniently expressed as the Enantiomeric Ratio ('E-value')[7] — the difference in the reaction rates (k_1, k_2) of the individual substrate enantiomers (*A*+*B*, Scheme 1) should be as large as possible. In an ideal process, where one enantiomer is transformed quickly and the other not at all, the reaction comes to a standstill at 50% conversion. In practice, however, such ideal cases are rare and E-values of most racemate resolutions are within the range 10–100. In such cases, the selectivity has to be enhanced,[8,9] which is feasible via modification of the catalyst, the substrate, or the 'environment', such as medium,[10] temperature,[11] pH, etc. Most of these selectivity-enhancement techniques have in common that the effects are generally not predictable since they are largely of empirical nature which requires trial-and-error experiments. In contrast to asymmetrization reactions, single-step racemate resolutions can also be optimized by terminating the reaction at an appropriate degree of conversion, albeit at the expense of chemical yield. Thus, the product is obtained in high e.e. below ~40% conversion, whereas the remaining starting material reaches its maximum e.e. beyond ~60% conversion.[7,12]

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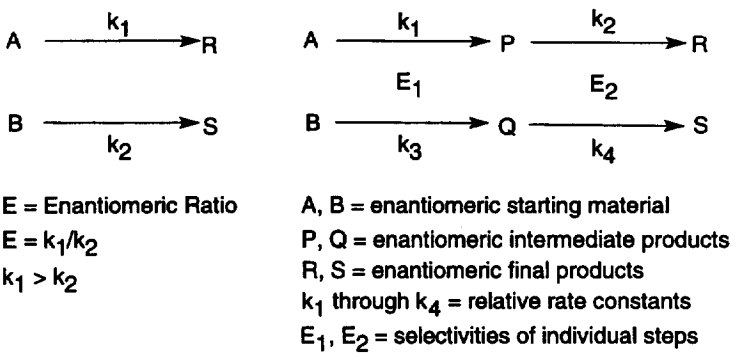

Scheme 1. Single-step and sequential kinetic resolution (Type-2 sequence).

One particular approach has proven to be very flexible (Scheme 1). When a bifunctional racemic substrate (*A*+*B*) carrying two reactive functional groups is involved, the reaction may proceed through two consecutive steps via intermediates *P*+*Q* to yield final products *R* and *S*. Both reactive groups may be chemically identical (but stereochemically different) but they may also be of different nature. As a consequence, the substrate has to visit the chiral catalyst twice which leads to a reinforcement of the chiral selection process. A powerful feature of this strategy is that both reaction steps are performed simultaneously in the same reaction vessel usually (but not necessarily) by the same catalyst.

The strength of sequential resolutions lies in the fact that the selectivitites of both steps may contribute to each other by accumulating 'mistakes', which occurred during the chiral selection process due to imperfect selectivities, in one of the three species involved — substrate (*A*+*B*), intermediate (*P*+*Q*) or final reaction product (*R*+*S*). Due to the fact that the product from the first resolution (*P*+*Q*) represents the substrate for the second, the overall kinetics become very complex. Whereas the kinetics of single-step kinetic resolutions are well understood and easily applied with respect to the prediction of substrate- and product-e.e. as a function of the conversion,[7,12,13] the practical application of sequential resolutions is hampered by a lack of a simple procedure for the handling of the kinetics. This drawback has prompted us to develop computer programs 'SeKiRe' for the treatment of sequential kinetic resolutions.[14] The features of these along with selected case-studies are described in this paper.[15] By using these programs, it is possible to determine at which point of conversion each species — either the remaining substrate (*A*+*B*), the reaction intermediate (*P*+*Q*) or the final product (*R*+*S*) — can be harvested with a maximum in chemical and/or optical yield.

It has to be emphasized that the programs are not applicable to processes where the starting material consists of a more complex *meso*/DL-mixture.[16–20] It can be anticipated that such reactions require exquisite stereoselectivities, which cannot easily be found. Likewise, complex sequences proceeding through more than two consecutive steps[21] or via a combination of enantiotopic group and diastereotopic face selectivity[22] are out of scope. Similarly, single-step resolutions, where each of the substrate enantiomers is transformed by two different catalysts into a total of four different products ('Parallel Kinetic Resolutions') require a different approach.[23,24] However, the above mentioned processes are generally impeded by analytical problems due to the occurrence of a large number of structurally very similar species and it is not surprising that examples for these reactions are rare.

Applicability

Sequential kinetic resolution has been frequently employed, particularly in biocatalyzed processes. Most popular were the hydrolysis of *rac*-diesters[25–35] and the reverse reaction, i.e. esterification[27,36] or acyl-transfer involving the corresponding (±)-diols as substrates.[26,27,37–43] A related acyl-transfer procedure was successfully applied to the resolution of diamines.[44] All of these processes were performed with a single enzyme.

Sequential resolution processes where two different reactions were linked in tandem involving two (bio)catalysts have also been reported. Thus, lipase-catalyzed ester hydrolysis produced a primary alcohol, which was oxidized by an alcohol oxidase to yield the corresponding carboxylic acid.[23] In a similar fashion, (±)-methyl α-chloropropionate was hydrolyzed by carboxyl esterase NP to liberate enantiomerically enriched α-chloropropionic acid, which in turn was dehalogenated to yield lactic acid under the action of a dehalogenase.[45] The resolution of α-substituted nitriles was shown to proceed via two consecutive steps catalyzed by a nitrile hydratase — producing the corresponding carboxamide as intermediate — which was further hydrolyzed to the carboxylic acid by an amidase.[46,47] In a related sequence, *rac*-hydantoins were hydrolyzed to yield L-α-amino acids by a hydantoinase and an N-carbamoylamino acid amidohydrolase through the corresponding intermediate N-carbamoyl amino acid derivative.[48] Alternatively, L-α-amino acids were obtained from N-acylamino acid esters through the action of a protease (α-chymotrypsin or subtilisin) — catalyzing the ester hydrolysis — and an aminoacylase, which cleaved the N-acyl group from the N-acylaminoacid intermediate.[49] The majority of the above mentioned sequential resolutions have in common that any attempts to make use of the potential amplification of selectivity have been neglected. Limited optimization by trial-and-error was mentioned rather scarcely[42,44] and only few studies were aiming at the detailed investigation of the kinetics.[23,35,36,45]

One subtype of sequential resolutions deserves special attention. Although on a first glimpse two reactive groups in the substrate would be considered necessary for a two-step process, compounds with a single reactive group may be subjected to sequential resolution if one considers a forward–reverse reaction sequence, e.g. ester hydrolysis–esterification,[50,51] ester hydrolysis–acyl transfer[52] or ester alcoholysis–acyl transfer.[53] Such processes can also be analyzed by using the program presented in this paper as long as the reactions analyzed are strictly irreversible.

Computer program features

Abbreviations

A and *B*=substrate enantiomers, *P* and *Q*=intermediate product enantiomers, *R* and *S*=final product enantiomers; E_1 and E_2=selectivity constants for step one and step two (Enantiomeric Ratio); E_T=selectivity constant for the sequential resolution;[54] *s*=specificity constant for substrates *A*+*B* and *P*+*Q*, corresponding to the ratio of the reaction rates of step one and step two [$s=(k_1+k_3)/(k_2+k_4)$]; k_1 through k_4 (k_i)=first-order rate constants; t_i=time; *c*=conversion, defined as the ratio of $(R+S)/(A_0+B_0)$; e.e.$_{A/B}$, e.e.$_{P/Q}$, e.e.$_{R/S}$=enantiomeric excess of substrate and products (positive and negative values indicate that enantiomers *A*, *P*, *R* or *B*, *Q*, *S* are in excess, respectively).

Analysis

Sequential irreversible kinetic resolutions can be analyzed based on experimental data and the four first-order rate constants (k_i) governing the kinetics of the process (Scheme 1). The following experimentally determined data can be used: substrate concentration [A_0] and [B_0] at start (set to a ratio of 1:1 by default, since *A*+*B* is usually a racemate, but this value can be varied, if desired); and [*P*] and [*Q*] at a given time (t_i). Alternatively, [*P*] and [*Q*] can be replaced by [*R*] and [*S*], if the latter species are easier to determine experimentally. Although a single data set would be mathematically sufficient for the calculation of the k_is, it should be pointed out that at least three but preferably four to five sets are recommended in order to obtain reliable results. This limits inaccuracies emerging from analytical procedures and deviations of the (actual) kinetics from the (theoretical) assumptions, e.g. due to inhibition, etc. (see below). This option provides an overall picture of the process and allows predictions on the function of the e.e.s and/or chemical yields on the conversion of the reaction.

Simulation

Starting from assumed or calculated first-order rate constants, the following twelve parameters can be plotted versus time or versus conversion (defined as $[R+S]/[A_0+B_0]$): concentration of all species

(i.e. [*A*], [*B*], [*P*], [*Q*], [*R*], [*S*], [*A*+*B*], [*P*+*Q*], [*R*+*S*]), and their enantiomeric composition (e.e.$_{A/B}$, e.e.$_{P/Q}$, e.e.$_{R/S}$) plotted as a function of time or conversion.

Based on the above assumed rate constants, single sets of data containing the above variables at a given moment (t_i) or point of conversion (c) can be obtained by using the 'single value' option.

This feature is designed for the modification of a given process through alteration of the k_is, e.g. by acceleration or deceleration of individual reactions via variation of the reactions conditions.[55] Due to mathematical reasons, cases where k_1 and k_2 and/or k_3 and k_4 equal each other cannot be handled, because (k_1-k_2) and/or (k_3-k_4) become nil. As a consequence, this would lead to division by zero. However, this case is not very likely, since in practice the k_is will deviate from each other at least by a very small value, thus avoiding this problem. If such cases should be simulated, the respective k_is should be chosen with a slight deviation from being equal (e.g. k_1=1000, k_2=999, etc.; for example, see Cases II, IV and VI).

Optimization

Based on the four relative rate constants which were either obtained from experimentally determined data (*Analysis*) or assumed (*Simulation*), the maximum obtainable e.e.$_{P/Q}$ (either *P* or *Q* being in excess) can be calculated with matching data for t_i, e.e.$_{A/B}$, e.e.$_{R/S}$, [*P*], [*Q*] and e.e.$_{P/Q}$. By using this option, the optimum point of harvest in a given process can be determined.

Theory

General remarks

The following assumptions were made.

(1) The specific activity of the enzyme remains constant during the whole period of the reaction, implying that no enzyme deactivation caused by pH, temperature, chemical or mechanical stress occurs.
(2) Absence of inhibition.
(3) Spontaneous (non-biocatalyzed) reactions can be neglected.
(4) All reactions are irreversible.
(5) For the *Analysis* option, the substrate must always be in excess.

Mathematics

In the derivations elaborated by Guo *et al.*[36] the k_is are relative second-order rate constants, since the concentration of the enzyme was also taken into consideration. The latter parameter, however, was reduced during further derivations. On the basis of our assumptions (see general remarks), it is possible to combine these second-order rate constants with the enzyme–substrate complex concentration to obtain pseudo first-order rate constants. Since for practical applications the relative rate constants (k_i) are more meaningful than the corresponding Michaelis–Menten constants — the former immediately provide a picture of the selectivities at a first glance — this program was written for the calculation of the values of all k_i. As a consequence, the system can be described as follows. For the sake of simplicity, the equations shown below have been derived for the transformations of $A \rightarrow B \rightarrow R$. The mathematics for the reactions $B \rightarrow Q \rightarrow S$ were obtained in an analogous way.

$$\frac{dA}{dt} = -k_1 A \tag{1}$$

$$\frac{dP}{dt} = k_1 A - k_2 P \tag{2}$$

$$\frac{dR}{dt} = k_2 P \tag{3}$$

After integration of Eqs 1–3 — with the assumption that the concentration of A is A_0 and the concentration of all other components (P, R) is nil at the beginning of the reaction (t=0) — Eqs 4–6 are obtained, where A, P and R are the respective concentrations at time t.

$$A = A_0 e^{-k_1 t} \tag{4}$$

$$P = \frac{A_0 k_1}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t}) \tag{5}$$

$$R = \left(1 + \frac{k_1 e^{-k_2 t} - k_2 e^{-k_1 t}}{k_2 - k_1}\right) A_0 \tag{6}$$

Since the sum of all concentrations is constant, it is also possible to write

$$S = B_0 - B - Q \tag{7}$$

Calculation of the time when P has an optimum: to obtain the optimum for P, the derivation of Eq. 5 versus time (dP/dt) has to be zero. The concentration of P has a maximum after a time of $t_{P\text{-opt}}$

$$t_{P-\text{opt}} = \frac{1}{k_1 - k_2} \ln \frac{k_1}{k_2} \tag{8}$$

Conversion: the conversion has been defined as the ratio of (R+S) to (A_0+B_0).

Enantiomeric excess: the program allows negative values for the e.e. to indicate which enantiomer is in excess. Thus, positive e.e.-values indicate that A, P and R are in excess, and for negative values B, Q and S are dominating. The following equation is used

$$\text{e.e.}_{A/B} = \frac{A - B}{A + B} \tag{9}$$

Limits: in order to construct diagrams, it is necessary to calculate the e.e.-values for the time approaching zero. By using the rules of l'Hôpital one obtains

$$\lim_{t \to 0} \text{e.e.}_{P/Q} = \frac{A_0 k_1 - B_0 k_3}{A_0 k_1 + B_0 k_3} \tag{10}$$

$$\lim_{t \to 0} \text{e.e.}_{R/S} = \frac{A_0 k_1 k_2 - B_0 k_3 k_4}{A_0 k_1 k_2 + B_0 k_3 k_4} \tag{11}$$

Calculation of k_is: the program allows the determination of all four k_is by using the following input data:

(a) time (t), and the concentrations of [A], [B], [P], [Q]; or

(b) time (t), and the concentrations of [A], [B], [R], [S].

The final set of data should be measured before the ratio of the concentration of the faster reacting enantiomer versus the corresponding starting material reaches a value of ~0.7. Most reliable results were obtained by using three to four individual data sets at a conversion below ~40%. Although it is obvious that the input data of (b) can easily be transformed into the input data of (a) and vice versa, two different ways are followed in each case to avoid errors caused by rounding values.

In contrast to Type-1 sequences,[15] the k_is of Type-2 processes are obtained in a much simpler and more exact way, since it is not necessary to search for a three-dimensional minimum. For all calculations the principle of the minimum of the sum of the errors squared is used.

Constants k_1 and k_3 are obtained using a simple linear regression, k_2 is obtained as the minimum of the error function F, with F standing for the sum of errors squared. In case the input data set (a) is used, Eq. 12 has to be zero otherwise Eq. 13 has to be zero.

$$\frac{dF}{dk_2} = 2 \sum \left\{ (P_{\text{calc.}} - P_{\text{meas.}}) \left[\frac{A_0 k_1 t e^{-k_2 t} - P_{\text{calc.}}}{(k_2 - k_1)} \right] \right\} \tag{12}$$

$$\frac{dF}{dk_2} = 2\sum\left\{(R_{calc.} - R_{meas.})\left[\frac{k_2e^{-k_1t} - k_1e^{-k_2t}}{(k_2 - k_1)^2} - \frac{k_1te^{-k_2t} + e^{-k_1t}}{k_2 - k_1}\right]\right\} \quad (13)$$

Constant k_3 was obtained by analogy. For $P_{calc.}$ see Eq. 5.

Case studies

The applicability of a given sequential resolution for preparative purposes — and the possibility to optimize such a process — is measured by two factors: (i) the chemical and (ii) optical yield. These factors are in turn determined by the rate constants k_1 through k_4, which provide the answer to the question of whether the reaction will be a success or not. In contrast to Type-1 reactions,[15] not only *P+Q* but also *A+B* and *R+S* can serve as the desired enantiomerically enriched product. The best choice depends on the kinetics of the process. By consulting the plots of chemical and optical yields versus e.e. generated from the program it can be determined which of the three species (*A+B*, *P+Q* or *R+S*) has to be harvested at which point of the reaction. The power of this method is illustrated by means of selected case studies which are representative for the merits and limits of the method.

Six different case studies were selected for the illustration of Type-2 sequential resolutions (Scheme 1). In four cases (Cases I–IV) the ratio of the reaction rate of the first step (k_1+k_3) versus the second step (k_2+k_4) is one by ten or vice versa. For cases V and VI, both steps were chosen to be equally fast since this latter phomenon is frequently encountered in practice. In a similar manner, the individual selectivites of each step (denoted as the Enantiomeric Ratios E_1 and E_2) are in the same range (E_1, E_2~10). For all cases, the lowest relative rate constant was arbitrarily set to 1. In general, it can be stated that the relative velocity of both steps has a major impact on the chemical yield of the intermediate product *R+S*. In analogy to Type-1 sequences, the latter can only be accumulated to a significant extent when the first reaction is considerably faster than the second. If this is not the case — i.e. step two is faster than step one — only *A+B* and/or *R+S* are the products of interest.

An interesting phenomenon is observed for the enantiocomposition of the intermediate product *P+Q*: depending on whether the enantiopreference of both steps is conserved — i.e. the enantiomer preferably formed in step one is also the one which reacts faster in step two ('parallel' selectivities, e.g. $k_1>k_3$ and $k_2>k_4$) — or vice versa ('anti-parallel' selectivities, e.g. $k_1>k_3$ and $k_4>k_2$) — and depending on the relative reaction rate of step one versus step two, the enantiomer of *P+Q* being in excess may switch during the course of the reaction (Cases I, III, IV and VI). This does not take place for *A+B* and *R+S*, where the same enantiomer is always in excess throughout the whole reaction.

In contrast to Type-1 sequences, where only one chiral product (*P+Q*) can be obtained, three species (*A+B*, *P+Q*, *R+S*) are now possible products of interest. In case the sequential resolution is to be performed in a stepwise manner — i.e. various products are harvested at different stages of the reaction — the following implications have to be kept in mind: only *R+S* can be removed from the reaction without disrupting the kinetics of the process as a whole because it represents the final product of an irreversible reaction. On the contrary, *A+B* and *P+Q* represent substrates for the first and second step, respectively, and their removal at any stage of the reaction would eliminate one (or both) steps and the process as a whole would be disrupted. As a consequence, the benefits of the selectivity-enhancement as a consequence of the double-sieving of the racemic substrate through two consecutive steps would be sacrificed.

Case I: k_1=*100*, k_2=*10*, k_3=*10*, k_4=*1*

The rate constants of Case I show a useful scenario for a sequential resolution of a racemate: The first step is faster than the second — *P+Q* is therefore accumulated to a considerable amount — and the selectivities are going in parallel. Thus, all of the species (*A+B*, *P+Q*, *R+S*) could be isolated at the appropriate stage of the reaction, each in about 35–40% chemical yield and ~90% e.e. However, a careful choice at which moment which species is harvested has to be made, since all of the species cannot be isolated in an accumulative manner for the reasons discussed above. Optimal results from a Case I reaction could be obtained in the following way: the final product *R+S* is isolated at an

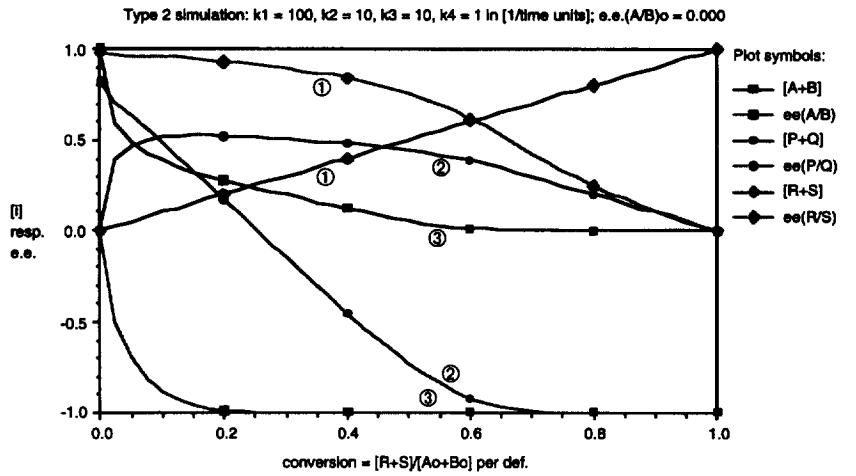

Figure 1. Case I, step one ten times faster than step two, parallel selectivities. (Negative e.e. values indicate *B*, *Q* and *S* being in excess.)

early stage of the reaction in ~35% yield and 90% e.e. with *R* being the predominating enantiomer (Figure 1, (1)). This would not lead to a disruption of the process. Then the reaction is continued until the e.e.$_{P/Q}$ has reached ~90% and the latter material can be isolated in ~40% yield. In this case, *Q* is the predominating enantiomer (2). At this point, ~25% of the starting material (*A*+*B*) remains, which consists of enantiopure *B*. Overall, this process yields both enantiomers in >90% e.e. with almost 100% chemical yield in total. It must be kept in mind that similar results could never be obtained via a single-step resolution showing comparable selectivities ($E \approx 10$).

Case II: $k_1=10$, $k_2=10$, $k_3=100$, $k_4=1$

For Case II, the enantiopreference of step two was inverted, i.e. opposite enantiomers are now preferred in each individual step. As a consequence, the e.e.$_{R/S}$ is now depleted as compared to Case I (Figure 2, (1)) and does not exceed a maximum value of ~55%, although its chemical yield remains the same since step one is still faster than step two. On the other hand, *P*+*Q* can be harvested in good optical and chemical yield at a late stage of the reaction (~30% yield, ~90% e.e. with *Q* in excess, (2)). Although *A*+*B* could be obtained in ~40% yield and ~90% e.e. at an early stage of the reaction (3), *P*+*Q* would be lost because this would disrupt the process.

Case III: $k_1=10$, $k_2=100$, $k_3=1$, $k_4=10$

Both of the following cases have in common that step two is slower than step one. As a consequence, the intermediate product *P*+*Q* is consumed faster than it is formed and its maximum concentration remains low throughout the whole reaction (<20%, Figure 3, (1)). On the other hand, *A*+*B* and *R*+*S* are of more interest in this case of parallel selectivities: Figure 3 shows that *R*+*S* can be isolated at about halfway of the reaction in ~40% yield and ~90% e.e. (with *R* being in excess, (2)). When the reaction is resumed, *A*+*B* is obtained with good results (~40% yield, ~90% e.e., *B* predominating, (3)).

Case IV: $k_1=10$, $k_2=10$, $k_3=1$, $k_4=100$

The worst-case scenario showing anti-parallel enantiopreference and step one being slower than step two is depicted in Figure 4. In comparison to Case III, the e.e.$_{R/S}$ is depleted and does not exceed a value of ~55% at about the halfway point of the reaction (1). Similarly, the concentration of *P*+*Q* remains low (max. ~20%) throughout the reaction and this species is therefore of no interest (2). Nevertheless, *A*+*B* can be isolated at a late stage of the reaction in ~40% yield and ~90% optical purity (3).

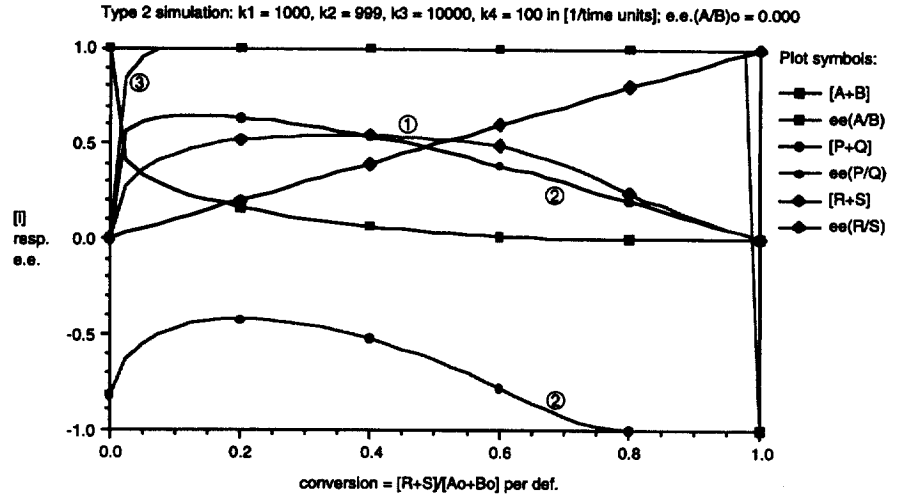

Figure 2. Case II, step one ten times faster than step two, anti-parallel selectivities. (Negative e.e. values indicate *B*, *Q* and *S* being in excess.)

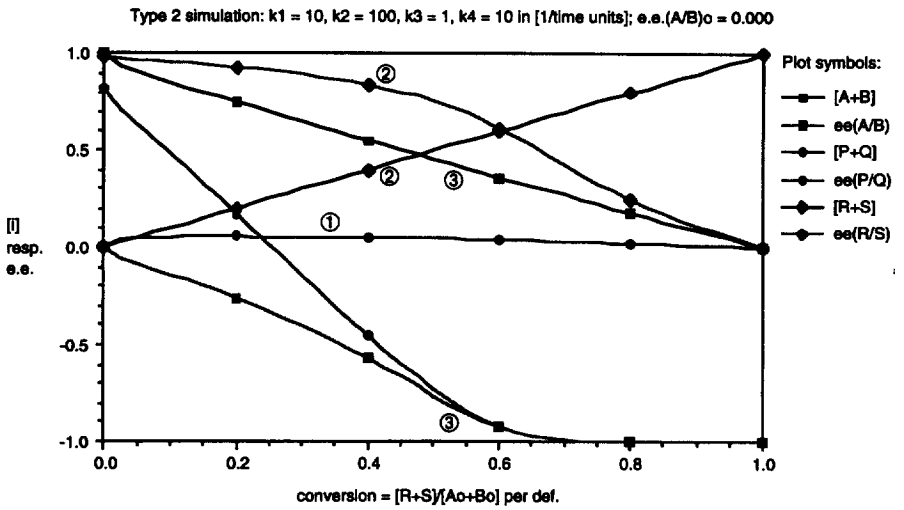

Figure 3. Case III, step one ten times slower than step two, parallel selectivities. (Negative e.e. values indicate *B*, *Q* and *S* being in excess.)

Case V: $k_1=10$, $k_2=1$, $k_3=1$, $k_4=10$

Cases V and VI represent a very common situation — both steps are equally fast. Due to the anti-parallel selectivities, *R+S* remains a racemate throughout the reaction and is therefore of no interest (Figure 5, (1)). On the other hand, the $e.e._{P/Q}$ is on a constant level of ~80% and this species can be accrued in ~45% yield at an early stage of the reaction with *P* being in excess (2). At this point, *A+B* can be harvested with an e.e. of ~90% in ~40% chemical yield (*B* in excess, (3)).

Case VI: $k_1=10$, $k_2=10$, $k_3=1$, $k_4=1$

Due to the parallel selectivities, *P+Q* is of no interest since its yield remains low throughout the whole process (Figure 6, (2)). However, at about halfway through the reaction, *R+S* (*R* predominating,

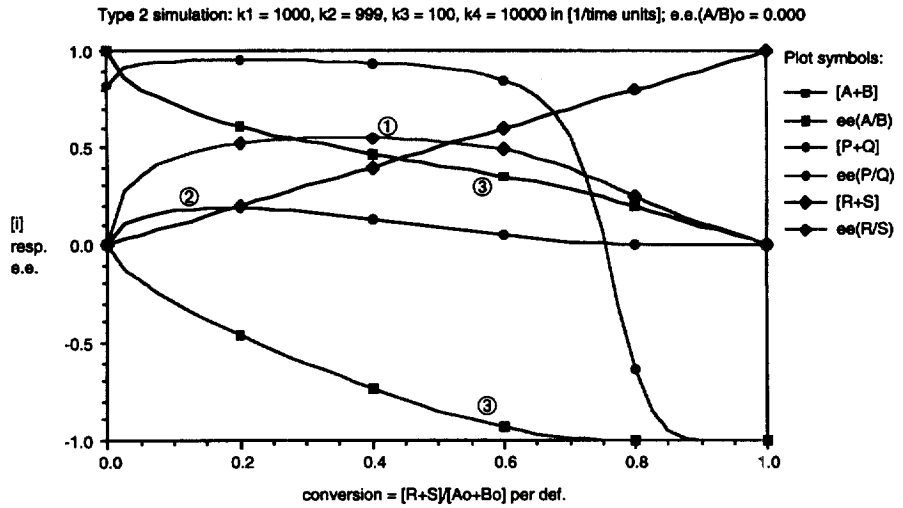

Figure 4. Case IV, step one ten times slower than step two, anti-parallel selectivities. (Negative e.e. values indicate *B*, *Q* and *S* being in excess.)

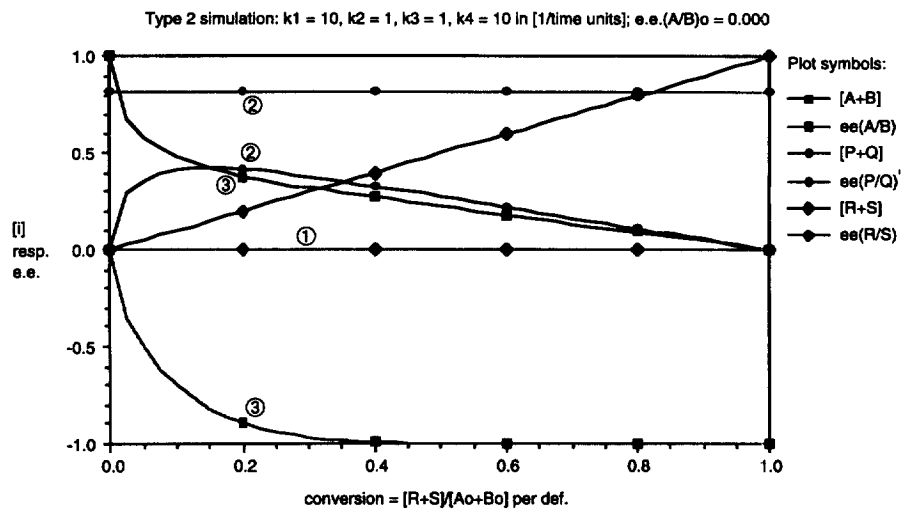

Figure 5. Case V, both steps equally fast, anti-parallel selectivities. (Negative e.e. values indicate *B*, *Q* and *S* being in excess.)

(1)) and *A*+*B* (*B* in excess, (3)) can be isolated in good optical (~90% e.e.) and fair chemical yield (~40% each).

The results from these case studies deserve special comment. Based on quantitative analyses and experimental data, several optimal scenarios which would lead to an optimal reinforcement of the enantioselectivities of the individual steps have been suggested so far. Thus, in sequential resolutions the relative rates of both steps should either be equal[55] or within a factor of about five[54] or, alternatively, at least within the same order of magnitude.[50] For such cases with both steps being equally fast, the overall efficiency of the process can be described as the total selectivity (E_T)[54]. In order to obtain comparable relative rates of step one and two, it is very difficult to modify the parameters by variation of the experimental conditions, in case (k_1+k_2) is significantly different from (k_3+k_4). However, in

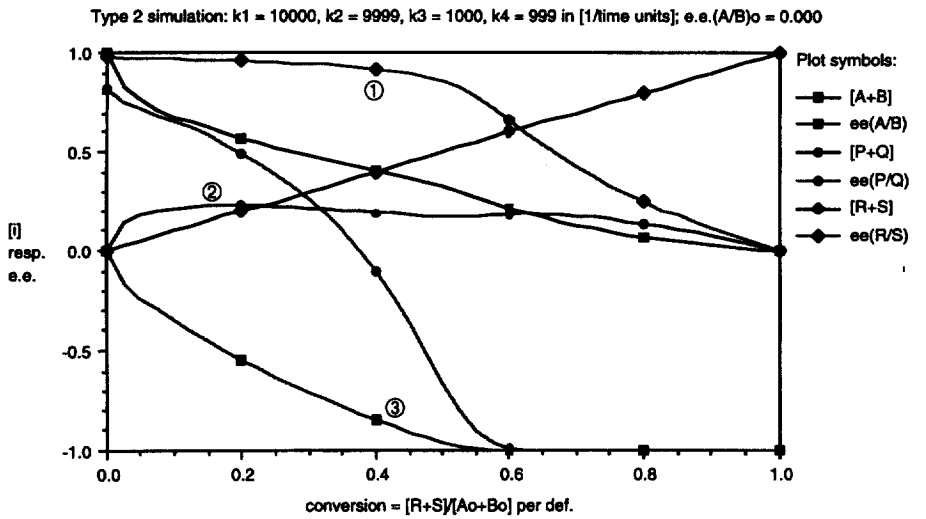

Figure 6. Case VI, both steps equally fast, parallel selectivities. (Negative e.e.-values indicate *B*, *Q* and *S* being in excess.)

selected cases this has been accomplished by addition of organic cosolvents[55] or by using two different enzymes at various concentrations.[45]

In extension to these general rules, it can be seen from Cases I–IV that sequential resolutions where the velocity of both steps are significantly different should not be disregarded *a priori*, but rather the best results out of a given situation can be obtained.

Summary

A computer program was developed for the treatment of sequential kinetic resolutions, i.e. kinetic resolutions which proceed via two consecutive steps. The program allows the analysis, simulation and optimization of such processes and it facilitates the determination at which point of the reaction which species — either the remaining substrate, the reaction intermediate or the final product — can be harvested with a maximum in chemical and/or optical yield. In contrast to conventional single-step processes, sequential resolutions offer considerably higher enantiomeric purities of product(s). In extension to previous studies recommending the rate of both steps being (near) equal to achieve an optimal reinforcement of the individual selectivities, it was shown that reactions which do not fit into this pattern can also lead to product(s) of acceptable e.e.s (≥90%) in reasonable chemical yields provided that the point of conversion for the harvest of the right species — either the remaining substrate (*A*+*B*), the reaction intermediate (*P*+*Q*) and/or the final product (*R*+*S*) — is carefully determined.

It is shown that in general the relative velocity of the first and the second reaction step has a major impact on the chemical yield of the intermediate, whereas the symmetry of the selectivites of both steps — either parallel or anti-parallel selectivities — determines the optical purities. The application of these programs for the optimization of sequential kinetic resolutions is being studied.

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