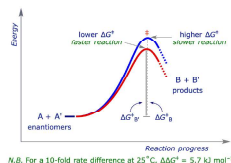
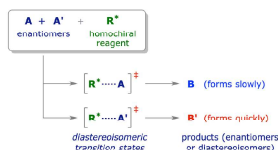
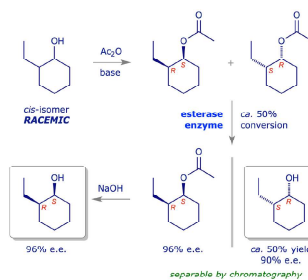


## Enzymatic or Kinetic Resolution

- When a racemic substrate reacts with a homochiral reagent two reactions occur, one with each enantiomer of the substrate.
- The transition states for the two processes have a **diastereoisomeric** relationship, and the respective activation energies are different.
- Therefore the reactions proceed at **different rates**. If the rates of the two reactions are sufficiently different, the reaction can be used for separating the enantiomeric reactants.

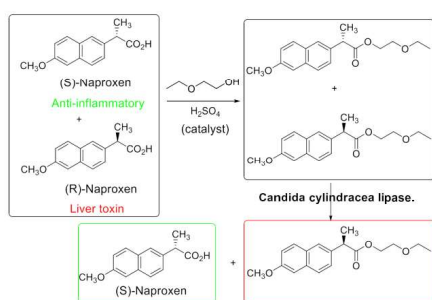


- There are some chemical processes which provide effective kinetic resolution, but the commonest examples involve the use of **enzymes**.
- Being themselves chiral, enzymes can discriminate between enantiomers,
- If the diastereoisomeric transition states formed when each enantiomer binds to the enzyme are significantly different in energy, the enantiomers will be 'processed' by the enzyme at different rates.



- Ester hydrolysis (or esterification) using an esterase enzyme can be particularly effective, proceeding with high selectivity under mild conditions and at various scales.
- It is one of the most commonly used kinetic resolution processes.

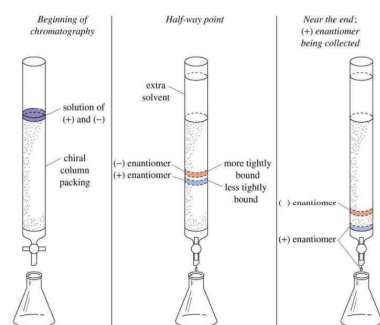
## Enzymatic or Kinetic Resolution



## Chiral Stationary Phases for Chromatographic Resolution

- A more versatile method for resolving a racemic mixture is through the use of **chromatography on chiral stationary phases**.
- These are applied in **gas chromatographic** and **liquid chromatographic** techniques.
- In the resolution of **racemic 2-aminobutane** on a chromatographic system, enantiomer of **mandelic acid** is attached to a stationary phase.
- Diastereomeric interactions between 2-aminobutane and the stationary phase lead to separable diastereomers with different retention times.

- As the solution passes through the column, the enantiomers form weak complexes, usually through hydrogen bonding, with the chiral column packing.
- These diastereomeric complexes have different physical properties.
- One of the two enantiomers will spend more time complexed with the chiral column packing.
- The solvent flows continually through the column, and the dissolved enantiomers gradually move along.
- Compounds that are adsorbed strongly spend more time on the stationary particles
- They come off the column later than less strongly adsorbed compounds, which spend more time in the mobile solvent phase.

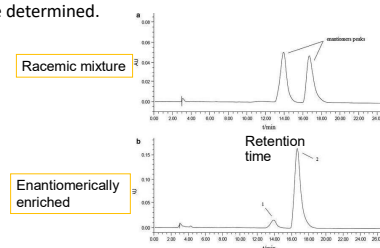


Chromatographic resolution of enantiomers. The enantiomers of the racemic compound form diastereomeric complexes with the chiral material on the column packing. One of the enantiomers binds more tightly than the other, so it moves more slowly through the column.

## Assessing the Efficacy of a Resolution

- Using a chromatogram of the resolved compound on a chiral stationary phase, the percentage composition can be determined.

- The efficacy of the different strategies for separating a racemic mixture can be determined by assessing the purity of each of the enantiomers obtained.
- The purity of each of the enantiomers is usually expressed as enantiomeric excess (ee).



## Calculating Enantiomeric excess (ee) by Percentage

- Enantiomeric excess (optical purity) is a measure of how pure an enantiomer is (i.e. the extent to which one enantiomer is present in excess of the **racemic mixture**).
- It is denoted by the symbol *ee* and calculated as a %.

$$ee = \% \text{ of major enantiomer} - \% \text{ of the minor enantiomer}$$

## Enantiomeric excess

- If the percentages of each of the two enantiomers in the mixture is calculated from the area under the peak in a chromatogram, the enantiomeric excess can be calculated directly using the formula below.
- Consider the case of a mixture containing 95% of one enantiomer and 5% of the other, the enantiomeric excess of the mixture is

$$95\% - 5\% = 90\%$$

## Calculating Enantiomeric excess (ee) by Mass

- The enantiomeric excess (purity) of a mixture can be calculated using the masses of each of the enantiomers in the mixture.
- If the masses of each of the pure enantiomers isolated after separation is known, the enantiomeric excess can be calculated using the equation below

$$ee = \left( \frac{R - S}{R + S} \right) \times 100$$

## Calculating Enantiomeric Excess (Assignment)

a) If (S)-mandelic acid has a specific rotation of +154 degrees then its enantiomer has a specific rotation of -154 degrees. As the specific rotation of the mixture is negative, (R)-mandelic acid is the dominant one.

b)

$$ee = ([\alpha]_{\text{mixture}} / [\alpha]_{\text{pure enantiomer}}) \times 100$$

$$ee = (75 / 154) \times 100 = 48.7\%$$

**ÖRN:** (R)-(-)-2-Bütanol ve (S)-(+)-2-Bütanol karışımı içeren bir örnek polarimetrede ölçülüyor. Gözlenen özgül çevirme +6,76° olarak ölçülüyor. Buna göre, optikçe saflık nedir? (S)-(+)-2-Bütanol'ün özgül çevirmesi +13,52°'dir.

**ÇÖZÜM:**

$$ee = ([\alpha]_{\text{mixture}} / [\alpha]_{\text{pure enantiomer}}) \times 100$$

$$= (6,76^\circ / 13,52^\circ) \cdot 100$$

=%50 (Kabın içinde bulunan (S)-(+)-2-Bütanol'ün fazlasıdır. Biri diğerinden bu kadar fazla demektir.

Yani geriye kalan % 50'nin % 25'i (R)-(-)-2-Bütanol ve % 25'i (S)-(+)-2-Bütanol demektir. Kabın içerisinde toplamda %75 (S)-(+)-2-Bütanol ve % 25 (R)-(-)-2-Bütanol olmalıdır.

### Homework

1. What is the enantiomeric excess of an adrenaline sample that has a specific rotation of -47.7°? Pure adrenaline has a specific rotation of -53.

2. How many percent of cholesterol and its enantiomer are present in a sample with an observed specific rotation of -22.4°? The specific rotation of pure cholesterol is -32°.

## Calculating Enantiomeric Excess (Assignment)

- The enantiomeric excess can also be calculated if the specific rotation  $[\alpha]$  of a mixture and the specific rotation  $[\alpha]$  of a pure enantiomer are known.

$$ee = ([\alpha]_{\text{mixture}} / [\alpha]_{\text{pure enantiomer}}) \times 100$$

- A sample of mandelic acid analysed in a polarimeter gave an observed specific rotation of -75 degrees. If the specific rotation of (S)-mandelic acid is +154 degrees;
  - Which enantiomer is in excess? (R or S)
  - Calculate the enantiomeric excess of the mixture.
  - Calculate the percentage of each enantiomer in the mixture.

c)  $R + S = 100 \rightarrow S = 100 - R$

$$ee = \% \text{ of major enantiomer} - \% \text{ of the minor enantiomer}$$

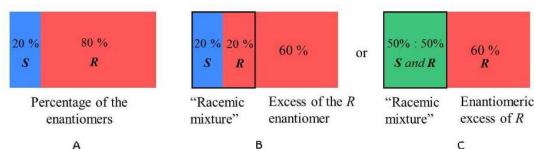
$$48.7 = R - (100 - R)$$

$$2R - 100 = 48.7$$

$$2R = 148.7$$

The major R-enantiomer = 74.4%

The minor S-enantiomer =  $(100 - R) = 25.6\%$



**Figure A** represents the percentage of each enantiomer. There is 20% of the S and 80% of the R enantiomer in the mixture. However, the **ee** of the R enantiomer is not 80% – it is only 60% as the other 20% of the R enantiomer makes up the *quasi-racemic* mixture with the 20% of the S enantiomer (**Figure B**).

**Figure C** shows this part of the mixture as one in green and what is left in the red part is the enantiomeric excess of the R enantiomer.