Bioanalytics

Part 5

12.11.2010

Chirality and Enantiomers

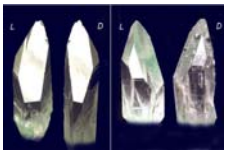
- Definition: Optical activity
- Properties of enantiomers
- Methods:
  - Polarimetry
  - Circular dichroism
- Nomenclature
- Separation of enantiomers
- Determination of enantiomeric excess (ee)
  Determination of absolute configuration
- Enantiomeric ratio (E-value)

Definitions

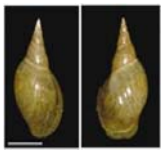
Enantiomers: the two mirror images of a molecule

Chirality: non-superimposable mirror-images

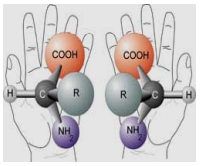
- Depends on the symmetry of a molecule
- Point-symmetry: asymmetric C, Si, S, P-atoms
- Helical structures (protein α-helix)



Quarz crystals



snail-shell

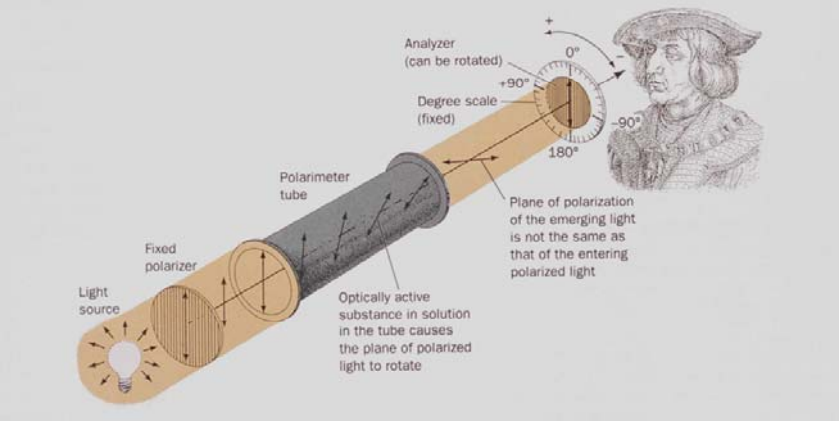


amino acids

Properties of Enantiomers

- Chemical identical
- Identical UV, IR, NMR-Spectra
- Differences:
  - Absorption and refraction of circular polarized light is different
    - Polarimetry, CD-spectroscopy
  - Interaction with other chiral molecules/surfaces is different
    - Separation of enantiomers on chiral columns (GC, HPLC)

Chiral compounds show optical activity



A polarimeter is a device which measures the angle of rotation by passing polarized light through an „optical active“ (chiral) substance.

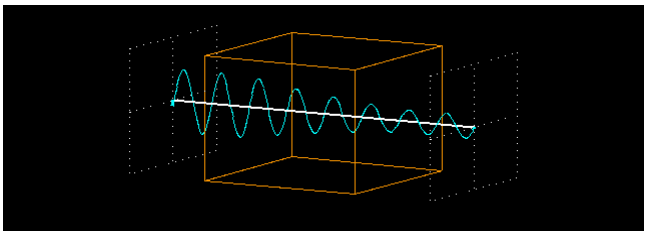
Interaction of light and matter

If light enters matter, its intensity (amplitude), polarization, velocity, wavelength, etc. may alter.

The two basic phenomena of the interaction of light and matter are *absorption* (or *extinction*) and a *decrease in velocity*.

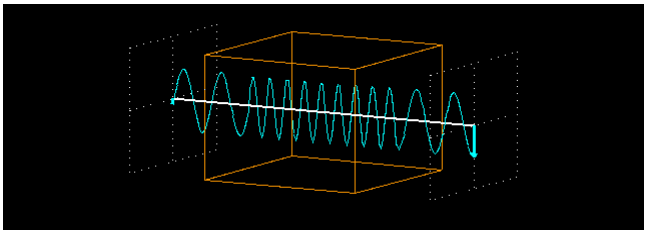
Interaction of light and matter

Absorption means that the intensity (amplitude) of light decreases in matter because matter absorbs a part of the light. (Intensity is the square of amplitude.)



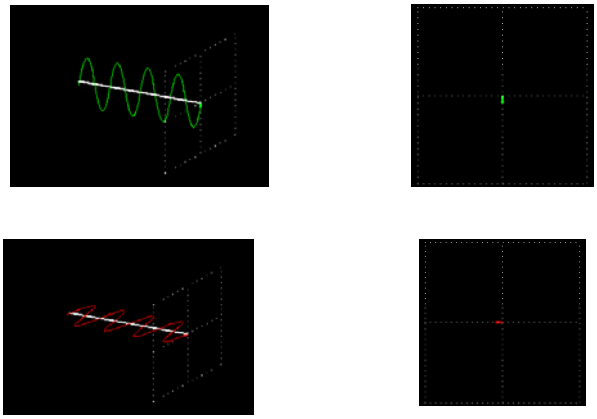
Interaction of light and matter

The **decrease in velocity** (i.e. the slowdown) of light in matter is caused by the fact that all materials (even materials that do not absorb light at all) have a **refraction index**, which means that the velocity of light is smaller in them than in vacuum. The refraction index is the ratio of the velocities of light measured in vacuum and in the given material.

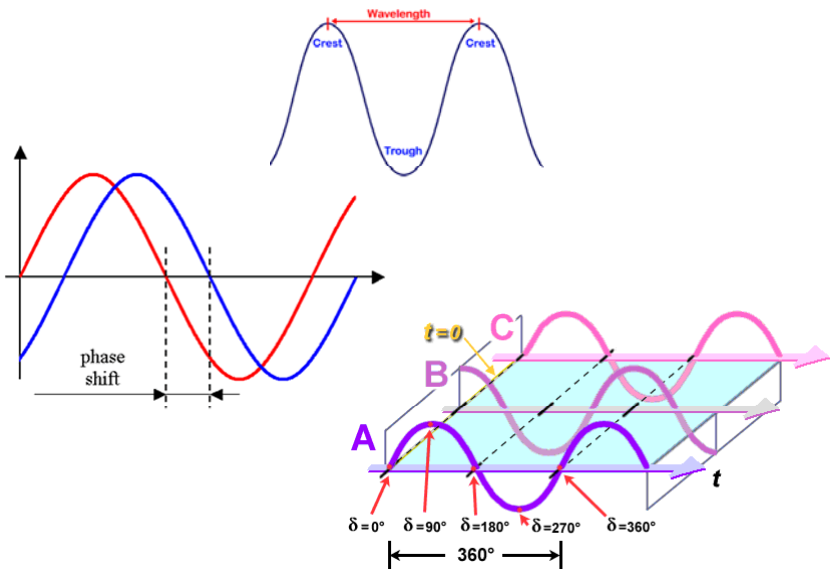


Refraction index >1

Definition: Linear-polarized light



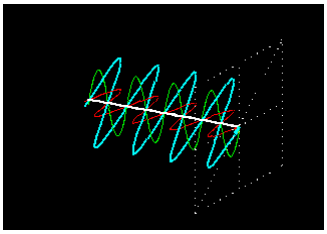
If the vector of the electric field (measured at a fixed point in space) oscillates along a straight line then the waves are called **plane-polarized** or **linearly polarized** waves.

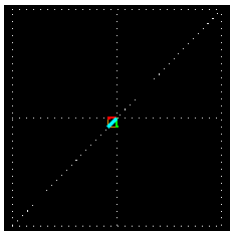


Schematic representation of phase in sine waves; t = time; waves 'A' and 'B' are 90° out-of-phase; waves 'A' and 'C' are in-phase.

Creation of linear polarized light

Superposition of plane-polarized waves in phase

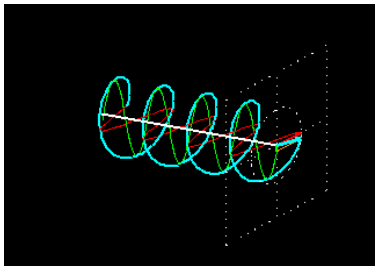


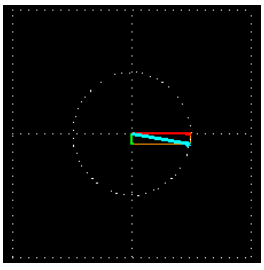


When two electromagnetic waves plane-polarized in two perpendicular planes are present simultaneously then the electric fields are added according to the rules of vector addition.
The properties of the resulting electromagnetic wave depends on the intensities and phase difference of the component waves.

Creation of circular polarized light

Superposition of plane-polarized waves out of phase

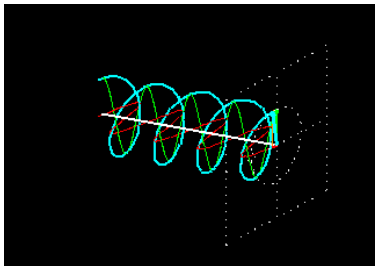


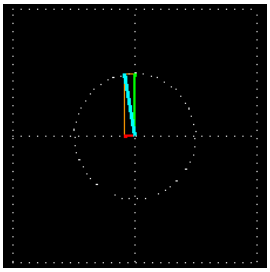


The superposition of two waves plane-polarized in two perpendicular planes with identical amplitude and a phase difference of 90° results in a right circular polarized wave.

Creation of circular polarized light

Superposition of plane-polarized waves out of phase

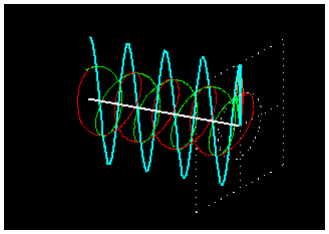


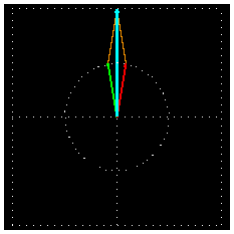


The superposition of two waves plane-polarized in two perpendicular planes with identical amplitude and a phase difference of -90° results in a left circular polarized wave.

Creation of linear polarized light

Superposition of left and right circularly polarized waves



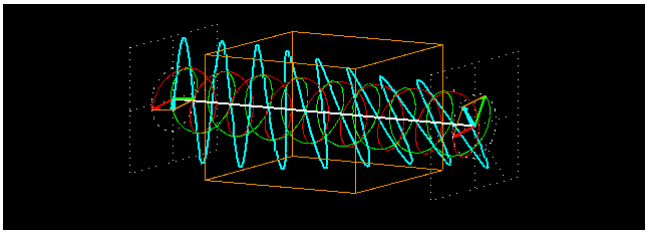


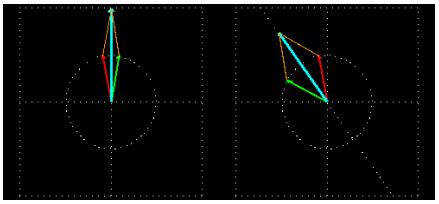
The result of superposing two circularly polarized waves (equal amplitude and wavelength) is a *plane-polarized wave*.

Any linearly polarized light wave can be obtained as a superposition of a left and a right circularly polarized light wave with identical amplitude.

Polarimetry

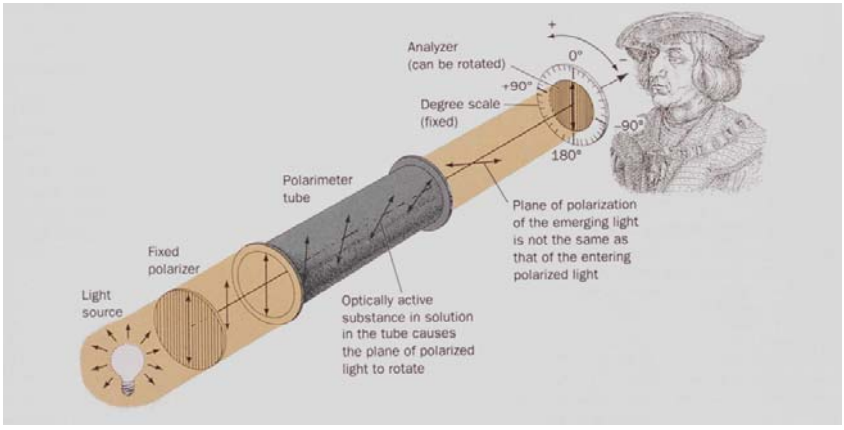
Polarimetry relys on differences in the refraction index for the two circular polarized fractions of a linear polarized beam. This phenomenon is called **circular birefringence** (double refraction).





As a result the polarization plane is rotated by the angle α.

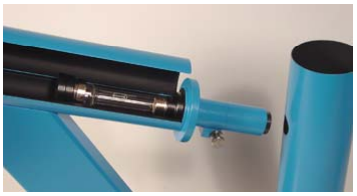
Polarimeter



A polarimeter is a device which measures the angle of rotation by passing polarized light through an optically active (chiral) substance.

Polarimetry

Half-shadow polarimeter



www.topac.com/polchem.html

Polarimetry

www.pharmacy.olemiss.edu/medicinal_chemistry/...

Polarimetry

As a result the rotation angle α is measured as specific rotation as a function of Temperature (25°C) and the wavelength (sodium-D-line = 589 nm):

$$[\alpha]_D^{25} = \frac{\text{observed rotation (degree)}}{\text{optical path length (dm)} \times \text{concentration } (\text{g} \cdot \text{cm}^{-3})}$$

L-alanine:

$\alpha_D^{20} = +14.5°$

L-(+)-alanine

L-phenylalanine:

$\alpha_D^{20} = -33.9°$

L-(-)-phenylalanine

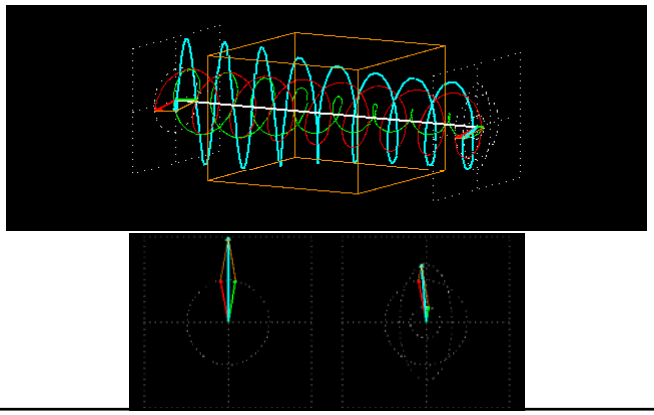
Circular dichroism

Definition: dichroism

- **Dichroism** means that the material absorbs two different types of light differently.
- **Simple dichroism**: a material absorbs light polarized in some plane differently than light polarized in a plane perpendicular to it.

Plane-polarized waves in a medium showing circular dichroism

Some materials possess a special property: *they absorb left circularly polarized light to a different extent than right circularly polarized light* (or vice verse). This phenomenon is called **circular dichroism**.



Application of CD

Circular dichroism (CD) spectroscopy is a type of absorption spectroscopy that can provide information on the structures of many types of biological macromolecules.

CD is measured as a quantity called
Mean residue weight ellipticity:

$$[\theta]_{MRW} = \frac{\psi}{10 \cdot c \cdot l \cdot n}\left[\deg \cdot cm^2 \cdot dmol^{-1}\right]$$

Molar ellipticity:

$$[\theta] = \frac{[\psi] \cdot M}{100}\left[10 \cdot \deg \cdot cm^2 \cdot mol^{-1}\right]$$

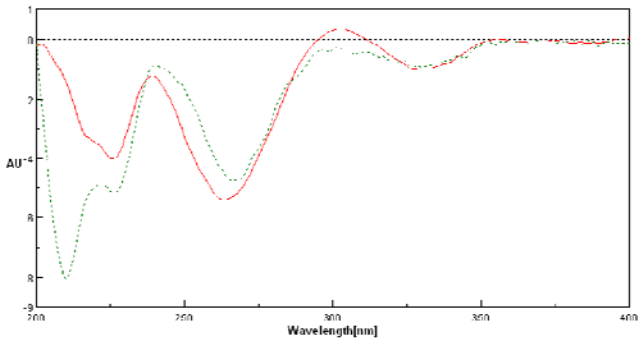
Specific ellipticity:

$$[\psi] = \frac{\psi}{c \cdot l}\left[10^{-1} \cdot \deg \cdot cm^2 \cdot g^{-1}\right]$$

c: concentration, l: pathway, n: number of amino acids

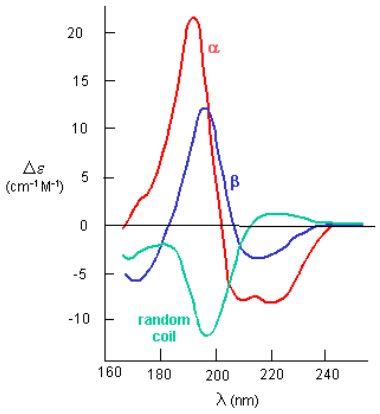
CD-spectra

Chiral compounds exhibit typical CD-signals with characteristic minima and maxima. Similar compounds exhibit similar spectra.



CD-spectra

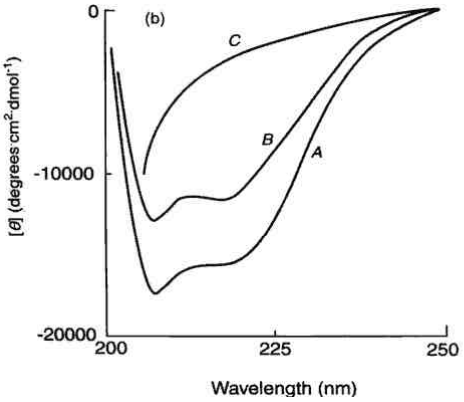
Far UV CD spectra associated with various types of secondary structure:



employees.csbsju.edu/.../ list_of_figures.htm

Protein unfolding monitored by CD

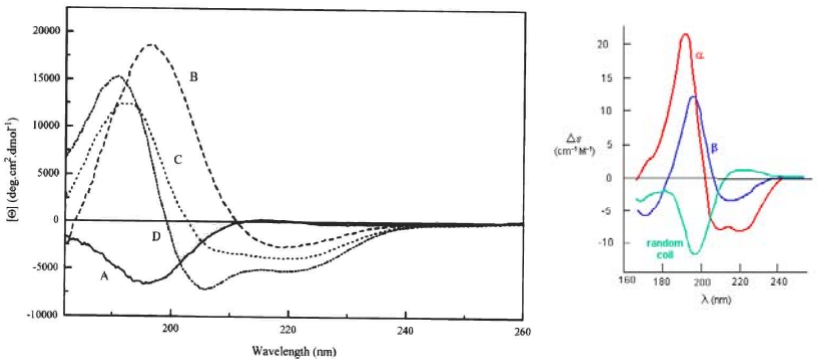
The unfolding of phosphorylase *b* by guanidinium hydrochloride (GdmCl) monitored by CD. (b) Far UV CD spectra recorded in the absence of GdmCl (A), and in the presence of 1 M (B) and 3 M (C) GdmCl.



Kelly & Price (2000) Current Protein and Peptide Science, 1, 349-384,*The Use of Circular Dichroism in the Investigation of Protein Structure and Function*

Structural switch monitored by CD

Far UV CD spectra of a 21 amino acid prion protein peptide.
Spectra were recorded in the absence of SDS (A), and in the presence of 3.5 mM (B), 7 mM (C) and 28 mM SDS (D) respectively.



Kelly & Price (2000) Current Protein and Peptide Science, 1, 349-384,*The Use of Circular Dichroism in the Investigation of Protein Structure and Function*

Worksheet 14 (5 min)

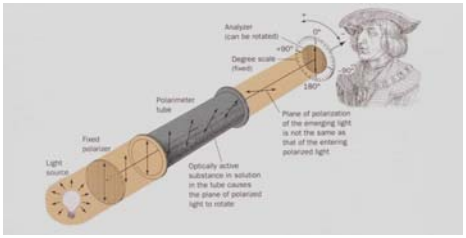
Nomenclature of Enantiomers

- +/- or d/l according to the specific rotation
- D/L-system (Emil Fischer)
- R/S-system (Cahn-Ingold-Prelog)

(+)/(-)-Nomenclature

The specific rotation α at (25°C) and (589 nm) is the oldest system of nomenclature to distiguish enantiomers:
(+): clockwise; (-): counterclockwise

In the old literature *d* (dextro = right)/ ***l*** (levo= left) refer to a clockwise (+) / counterclockwise (-) shift of the rotation plane.

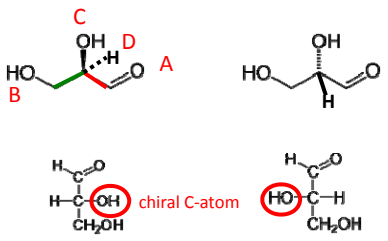


Note: The d / l-nomenclature is different from the D/L-Nomenclature!

D/L-Nomenclature: Fischer projection

D/L-System:

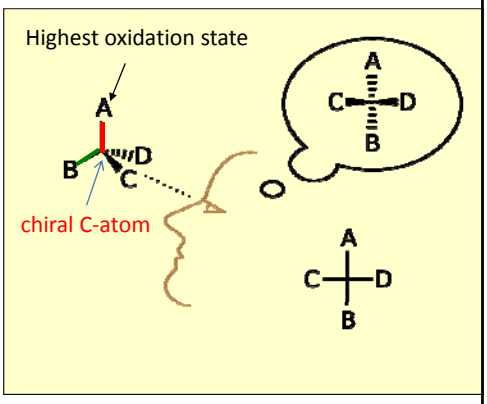
Used for sugars and amino acids
Reference system: glyceraldehyde



D-glyceraldehyde L-glyceraldehyde

OH-group on the right side OH-group on the left side

D= dextro, L= levo



Fischer projection: D/L-System

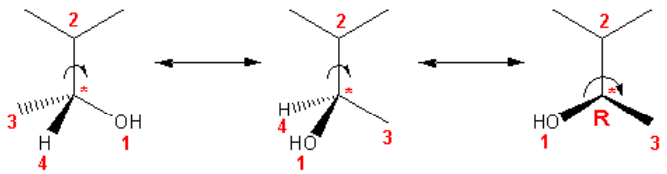
The D/L nomenclature is unrelated to (+)/(−); it does not indicate which enantiomer is dextrorotatory and which is levorotatory. Rather, it says that the compound's stereochemistry is related to that of the dextrorotatory or levorotatory enantiomer of glyceraldehyde—the dextrorotatory isomer of glyceraldehyde is in fact the D isomer.

Nine of the nineteen L-amino acids commonly found in proteins are dextrorotatory (at a wavelength of 589 nm), and D-fructose is also levorotatory.

R/S-(Cahn-Ingold-Prelog) Nomenclature

Priority of residues according to atom numbers:

-I > -Cl > -S-CH_3 > -SH > -F > -O-CH_3 > -OH > -N_3 > -$N(CH_3)_2$ > -NH-C_6H_5 > -NH_2 > -COOH > -CON_2H > > -$CONH_2$ > -CHO > -CH_2OH > -CD_3 > -CD_2H > -CDH_2 > -CH_3 > -D > -H > free electron pair



1. Number residues according to priority

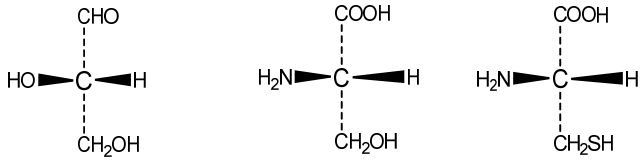
2. Turn residue with lowest priority away from observer

3. Determine sequence of residues 1-3: clockwise: *R*; conterclockwise: *S*

Note

The D/L-system and (R/S)-system are completely different systems of nomenclature.

Translation of D/L- into R/S-nomenclature

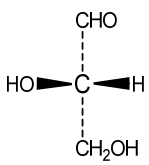


L-glyceraldehyde L-serine L-cysteine

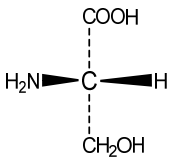
Number residues according to Cahn-Ingold-Prelog (atom numbers)

Worksheet 12 (5 min)

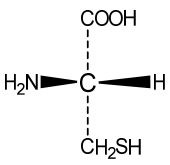
Translation of D/L- into R/S-nomenclature



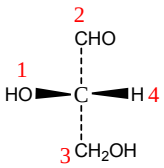
L-glyceraldehyde

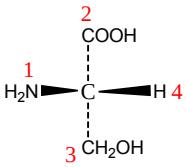


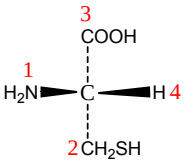
L-serine



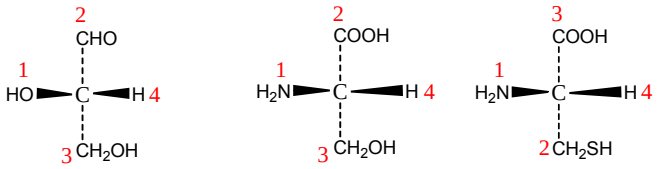
L-cysteine





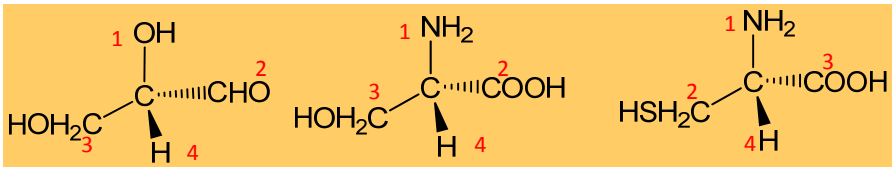


Translation of D/L- into R/S-nomenclature

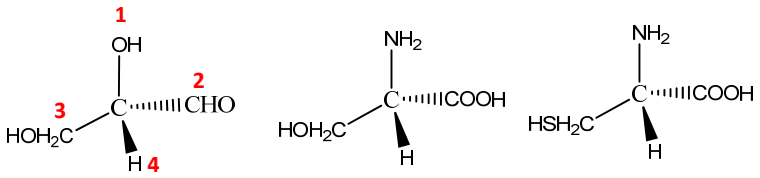


L-glyceraldehyde L-serine L-cysteine

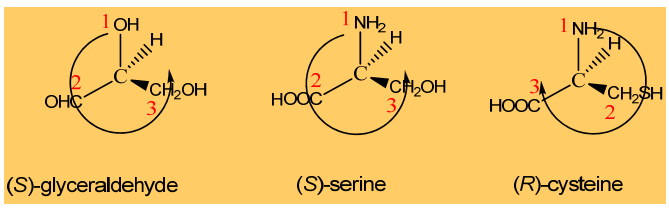
Draw tetrahedral structure with the residue of priority 1 on the tip.



Translation of D/L- into R/S-nomenclature



Turn residue with lowest prioritiy away from the observer.



(*S*)-glyceraldehyde (*S*)-serine (*R*)-cysteine

All natural L-amino acids are (*S*)-amino acids, with the exception of cysteine!

Note

Optical rotation and configuration (arrangement of substituents) at the chiral center are not correlated!

(*S*)-alanine = L-alanine:

$\alpha_D^{20} = +14.5°$

(*S*)-(+)-alanine

(*R*)-alanine = D-alanine:

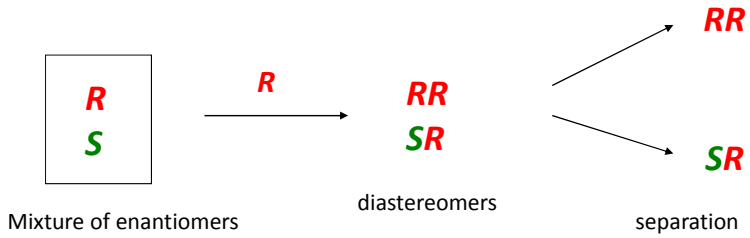
$\alpha_D^{20} = -14.5°$

(*R*)-(-)-alanine

Separation of enantiomers

As enantiomers have almost similar physical properties. One has to make them different to separate them.

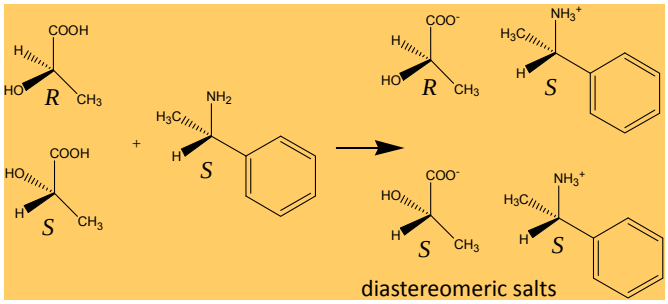
Formation of diastereomers (compounds with two stereo centers):



Diastereomers have different physical properties and can be separated e.g. by chromatography, crystallisation,

Separation of enantiomers

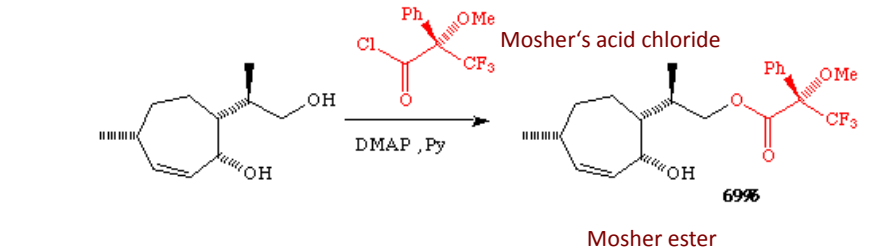
Separation of (*R,S*)-lactic acid by salt formation:

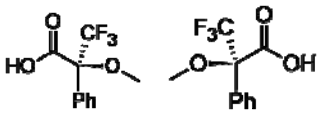


Diastereomeric salts have different crystallisation properties

Separation of enantiomers

Diastereomer formation by esterification:

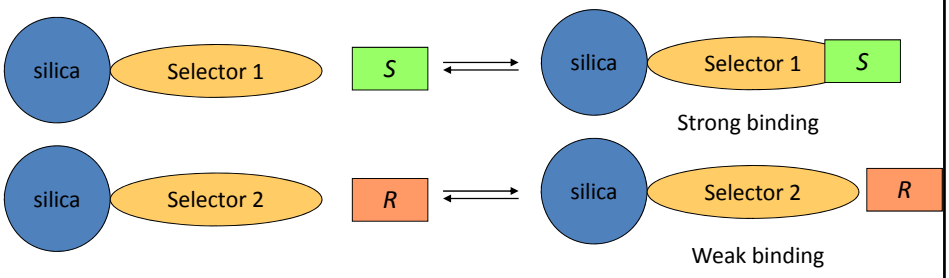




α-methoxy-α-trifluoromethylphenylacetic acid (MTPA)

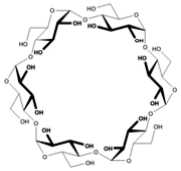
Separation of enantiomers

- Separation of enantiomers on chiral stationary phases (HPLC, GC)
- Separation is based on the formation of non-covalently bound diastereomers. The weaker diastereomer elutes earlier.



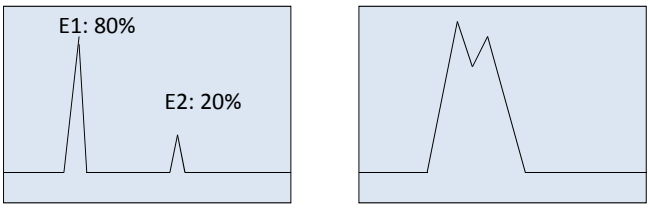
Separation of enantiomers

- Commonly, separations of enantiomers are performed as normal phase HPLC: on a hydrophilic stationary phase and a hydrophobic mobile phase.
- Chiral selectors are based on natural and synthetic polymers (e.g. polysaccharides, polyacrylamides, proteins)
  - Chiralcel: modified cellulose
  - Chiralpak: modified amylose
  - Chiradex, Nucleodex: modified cyclodextrines



Separation of enantiomers

- Baseline separation is essential to determine the enantiomeric excess



Enantiomeric excess

- The value of the enantiomeric excess can range from 0% to 100%.
- For a compound containing a single chiral center, a racemic mixture consists of 50% of the *R*-enantiomer and 50% of the *S*-enantiomer. The enantiomeric excess is 0.
- A sample that contains 100% of one enantiomer has an enantiomeric excess of 100%.

$$ee = \frac{[Enantiomer_1] - [Enantiomer_2]}{[Enamtiomer_1] + [Enantiomer_2]}$$

With the concentration of Enantiomer 1 > Enantiomer 2

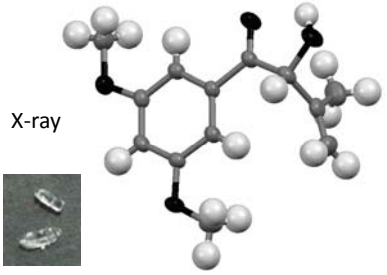
Worksheet 13 (10 min)

Determination of absolute configuration

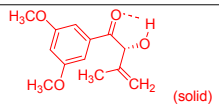
- Comparison with CD-spectra of similar compounds with known absolute configuration.
- Comparison of optical rotation with literature values
- NMR analysis of diastereomers e.g. Mosher esters
- X-ray spectroscopy of crystalline enantiomers

Absolute configuration: *R*

X-ray

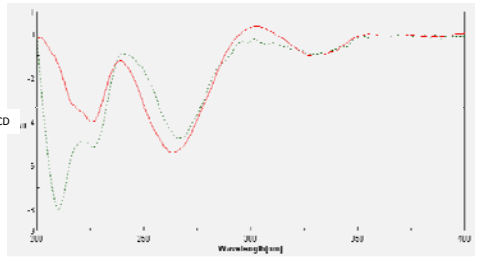


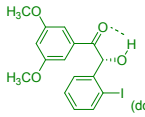
Absolute configuration determination required heavy metal atom replacement in the structure.



(solid)

(*R*)-1-(3,5-dimethoxyphenyl)-
2-hydroxy-3-methylbut-3-en-1-one





(dotted)

(*R*)-1-(3,5-dimethoxyphenyl)-
2-hydroxy-2-(2-iodophenyl)ethanone

P. Dünkelmann, D. Kolter-Jung, A. Nitsche, A. S. Demir, P. Siegert, B. Lingen, M. Baumann, M. Pohl, M. Müller, *Journal of the American Chemical Society* **2002**, *124*, 12084.

Carola Desen, AG Michael Müller, Uni Freiburg

E-value

- What is a E-value?
- How is it measured?

E-value = enantiomeric ratio

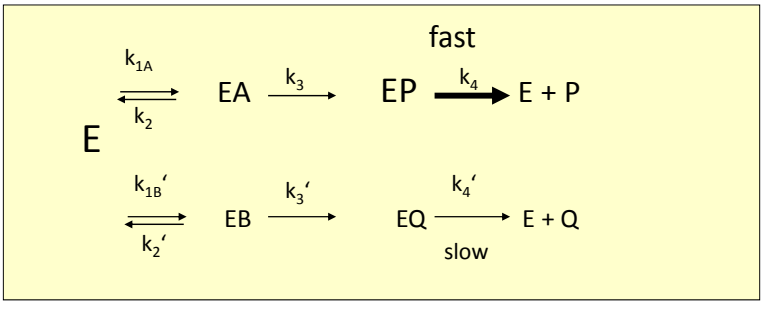
The E-value determines the enantioselectivity of a reaction.

This parameter is applied in chemistry and enzymology.

It is a measure for the ability of a catalyst to distinguish between enantiomers under defined conditions (pH, T, solvent…)

E-value

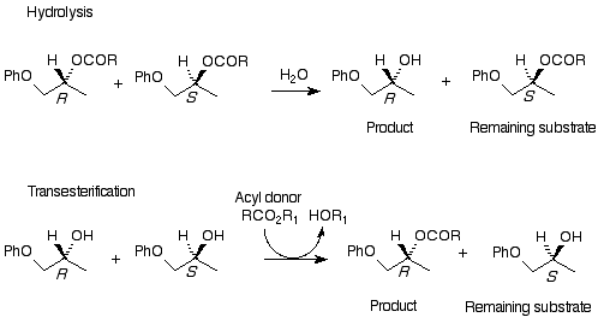
E-values are important, in reactions of enantiomers with chiral catalysts, e.g. in kinetic resolution



A, B: both enantiomers of the substrate;
P, Q: both enantiomers of the product
E: enzyme

Kinetic resolution: example

Highest selectivity for only one enantiomeric substrate is desired, however the less favored enantiomer is often also transformed.

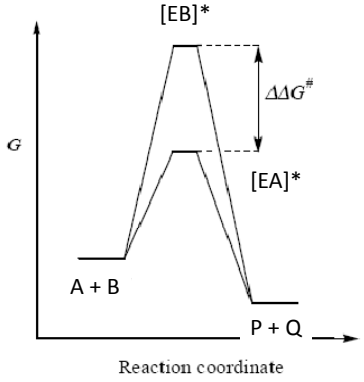


100 % selectivity: A + B → P + B

< 100% selectivity: A + B → P + Q + B

E-value

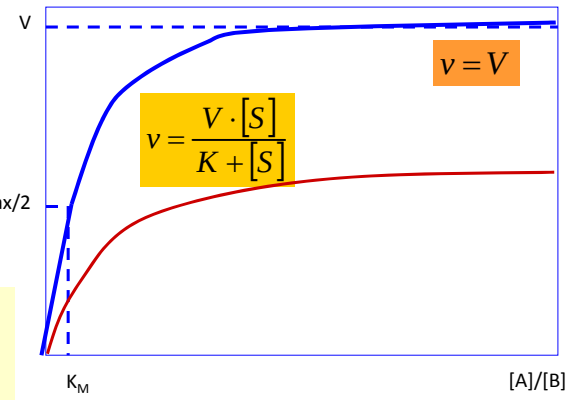
As chiral catalysts bind chiral substrates in a different way (one fits better than the other), the activation energy for both reactions is different, resulting in different reaction rates for both enantiomers.
By this kinetic principle, resolution of enantiomers is possible.



E-value

Different enantiomers show different K_M- and V_{max}-values

Zero order kinetics



app. 1st order kinetics

$$v = \frac{V}{K} \bullet [S]$$

E-value

For virtually irreversible reactions without product inhibition:

E-value can be calculated from the kinetic parameters for each enantiomer (if available).

$$v_A = \frac{V_A}{K_A} \bullet [A]$$

$$v_B = \frac{V_B}{K_B} \bullet [B]$$

$$\rightarrow \quad \frac{v_A}{v_B} = \frac{\frac{V_A}{K_A}[A]}{\frac{V_B}{K_B}[B]} = \frac{V_A}{V_B}\frac{K_B}{K_A}\frac{[A]}{[B]}$$

E

The E value is the proportion of reaction velocities for both enantiomers.

An E-value of 20 means that the initial rate for the reaction of one enantiomer is 20-times faster than for the other enantiomer.

E-value

$$v_A = \frac{V_A}{K_A} \bullet [A]$$

$$v_B = \frac{V_B}{K_B} \bullet [B]$$

$$\frac{v_A}{v_B} = \frac{\frac{V_A}{K_A}[A]}{\frac{V_B}{K_B}[B]} = \underbrace{\frac{V_A}{V_B}\frac{K_B}{K_A}}_{E}\frac{[A]}{[B]}$$

E is dependent on the ratio of the specific parameters V_{max} and K_M and independent on the substrate concentration.

E = 1: unselective

E > 20: acceptable

E > 30: very good

E>100: excellent

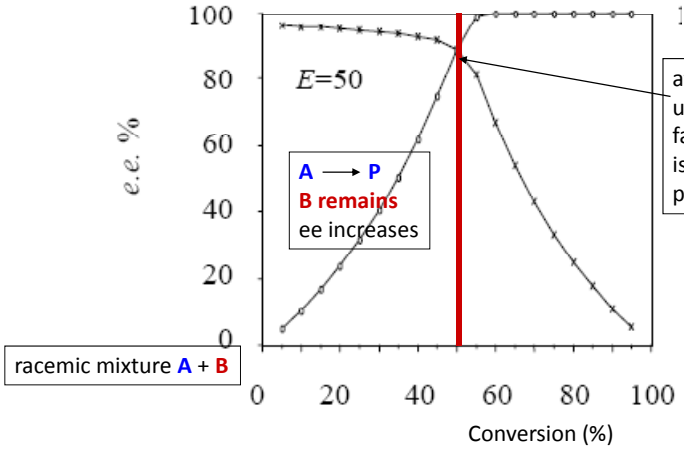
E-value, final ee and conversion

In a kinetic resolution, the enantiomeric purity of the product and the starting material varies if the reaction proceeds.

Thus, comparing *ee*-values of two kinetic resolutions is meaningful only at the same degree of conversion.

E-value, final ee and conversion

A + B ⟶ P + Q



racemic mixture A + B

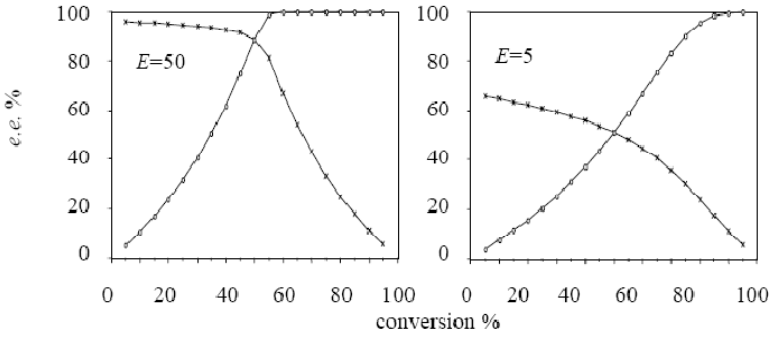
at 50% conversion A is used up and the less favoured enantiomer B is now transformed; the product ee decreases.

o: *ee* of the substrate; x: *ee* of the product

Worksheet 15 (10 min)

E-value, final ee and conversion

o: *ee* of the substrate; x: *ee* of the product



E=50 c = 50% ~90% ee
95% ee P = 30% conv.
95% ee S ~ 55% conv.

E=5 c = 50% ~50% ee
95% ee P not possible
95% ee S >80% conv.

Determination/calculation of E-values

If the pure enantiomeres of the substrate are not available it is not possible to calculate Vmax and K_M for each enantiomer, in such cases one can calculate E-values by measuring two of three variables:

- Enantiomeric purity of the substrate (ee_s)
- Enantiomeric purity of the product (ee_p)
- Extend of conversion (c)

For irreversible reactions the following equations are applied:

$$E = \frac{\ln[1 - c(1 + ee_p)]}{\ln[1 - c(1 - ee_p)]} \qquad E = \frac{\ln[(1 - c)(1 - ee_s)]}{\ln[(1 - c)(1 + ee_s)]} \qquad E = \frac{\ln\left[\frac{1 - ee_s}{1 + ee_s / ee_p}\right]}{\ln\left[\frac{1 + ee_s}{1 + ee_s / ee_p}\right]}$$

Further reading: K. Faber: Biotransformations in Organic Chemistry, Bommarius, Riebel: Biocatalysis

Determination of E-values

For irreversible reactions the following equations are applied:

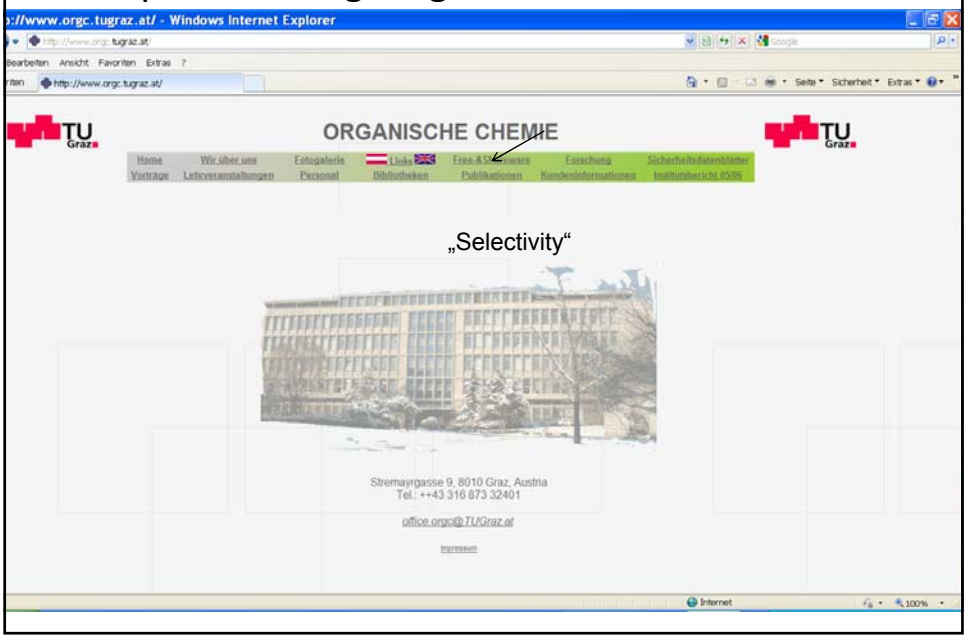
$$E = \frac{\ln\left[1 - c\left(1 + e.e._p\right)\right]}{\ln\left[1 - c\left(1 - e.e._p\right)\right]} \qquad E = \frac{\ln\left[(1-c)(1-e.e._s)\right]}{\ln\left[(1-c)(1+e.e._s)\right]} \qquad E = \frac{\ln\left[\frac{1-e.e._s}{1+e.e._s/e.e._p}\right]}{\ln\left[\frac{1+e.e._s}{1+e.e._s/e.e._p}\right]}$$

Prerequisites:

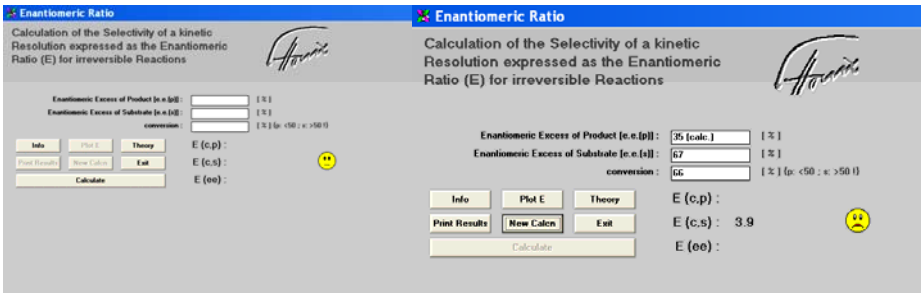
1. The separation of racemates is carried out in a homogeneous batch reactor
2. The reaction can be described as a (pseudo) uni-uni reaction
3. The reaction must obey a (pseudo) Michaelis-Menten mechanism
4. The reaction is irreversible
5. In case of equilibrium reactions the equilibrium constant has to be included

Further reading: K. Faber: Biotransformations in Organic Chemistry, Bommarius, Riebel: Biocatalysis

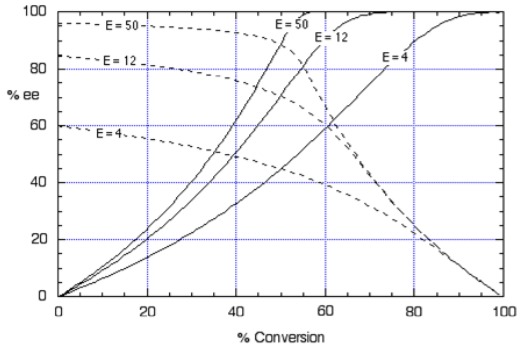
- http://www.orgc.tugraz.at/



Selectivity tool



E-value, final ee and conversion



The higher the E-value, the more the E-curves approach. This is due to the fact that E is a logarithmic function.
Thus, high E-values are less accurately measureable than low or moderate E-values.
E values > 50 can hardly be measured precisely.

E-value: conclusions

The E-value is a further characteristic parameter (such as specific activity, K_M) of a catalyst under defined reaction conditions.

The E-value is a logarithmic function. It correlates the different affinities and velocities a chiral catalyst has for two different enantiomers.

The higher the E-value, the higher is the selectivity of a catalysts.

An E-value = 30 does not mean, that the reaction with the preferred enantiomer is generally by a factor of 30 faster! This is only true under initial rate conditions.

E-value and enantiomeric excess (ee) are correlated by the conversion of the reaction as both the *ee* of the substrate and the product changes with conversion.

Next lecture:
November, 26
12-15 h