Spektropolarimetri

apt. Ihsanti Dwi Rahayu, S.Farm., M.S.Farm.

Farmasi Fakultas Kedokteran Universitas Lampung

Circular Dichroism (CD) Spectropolarimeter

Circular Dichroism-Optical Rotatory Dispersion (CD-ORD) Spectropolarimeter

Specifications

- Light source: 150W Xe lamp
- Monochromator: Double prism polarizing monochromator
- Wavelength range:
  - 163 – 950 nm (standard)
  - 163 – 1600 nm (option)
- Wavelength accuracy:
  - ±0.1 nm (163 to 250 nm)
  - ±0.2 nm (250 to 500 nm)
  - ±0.5 nm (500 to 800 nm)
  - ±1.5 nm (800 to 950 nm)
- Wavelength reproducibility:
  - ±0.05 nm (163 to 500 nm)
  - ±0.1 nm (500 to 800 nm)
  - ±0.5 nm (800 to 950 nm)
- Wavelength resolution: 0.025 nm
- Spectral bandwidth: 0.01 – 16 nm
- Slit width: 1 – 4000 um
- Scanning speed: max. 10000 nm/min
- CD full scale: ±8000 mdeg
- CD resolution: 0.00001 mdeg

Typical Conditions for CD

- Protein Concentration: 0.25 mg/ml
- Cell Path Length: 1 mm
- Volume 400 µl
- Need very little sample 0.1 mg
- Concentration reasonable
- Stabilizers (Metal ions, etc.): minimum
- Buffer Concentration : 5 mM or as low as possible while maintaining protein stability
- A structural biology method that can give real answers in a day.

Circular Dichroism (CD) Spectropolarimeter

- Circular Dichroism (CD) is an absorption spectroscopy method based on the differential absorption of left and right circularly polarized light.
- Optically active chiral molecules will preferentially absorb one direction of the circularly polarized light. The difference in absorption of the left and right circularly polarized light can be measured and quantified.
- It is mostly used to study biological molecules, their structure, and interactions with metals and other molecules.

Circular Dichroism (CD) Spectropolarimeter

- UV CD is used to determine aspects of protein secondary structure.
- Vibrational CD, IR CD, is used to study the structure of small organic molecules, proteins and DNA. UV/Vis CD investigates charge transfer transitions in metal-protein complexes

J-1700 (163 - 2500 nm)

J-1500 (163 - 1600 nm)

J-1100(180 - 600 nm)

FVS-6000

(2500 - 13333 nm)

(4000 - 750 cm^{-1})

Electronic Circular Dichroism	Vibrational Circular Dichroism

CLASSIFICATION..

- **Far UV-CD** (200nm-250nm) is used to investigate the secondary structure of protein. Change in protein secondary structure as a function of temp.(T) or conc.(C) can be known. Which also allows us to know..

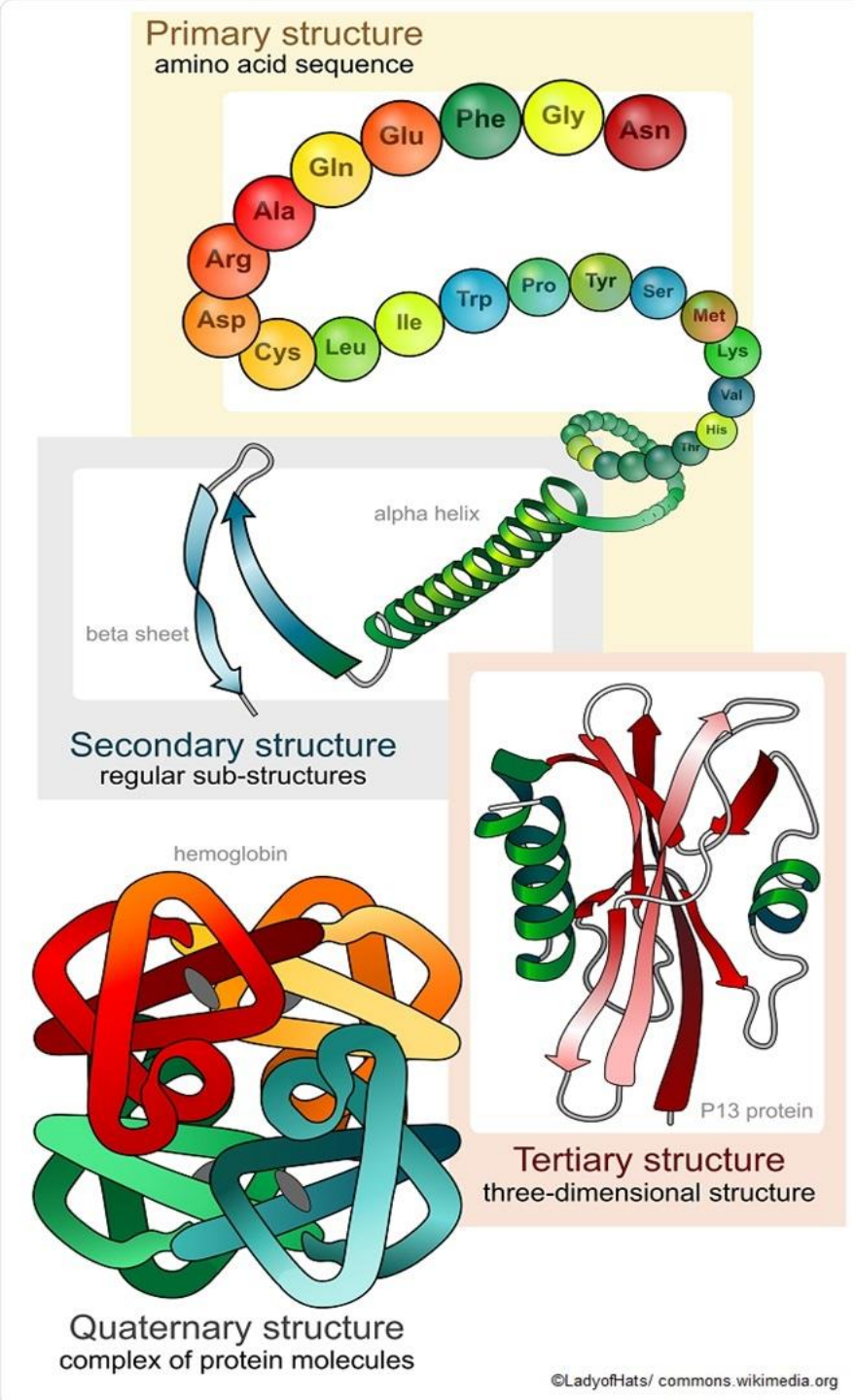
  1. Enthalphy
  2. Gibbs free energy of the process.

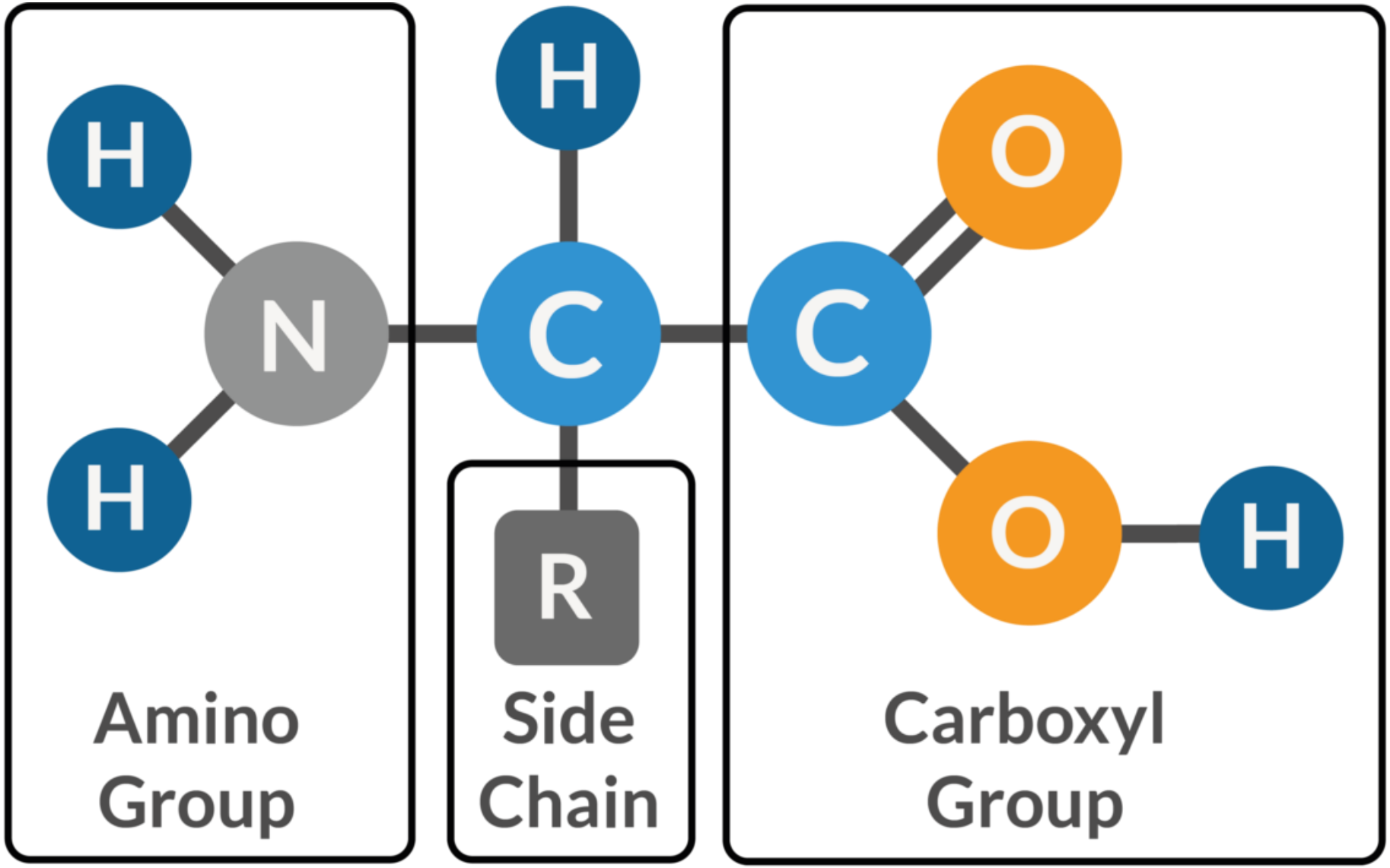
- **Near UV-CD** (250nm-350nm) spectra provides information on tertiary structure. It provides information on the nature of the prosthetic group present in a protein.
- **UV-Vis CD** (350nm-750nm) spectra in visible region is only found when metal is in chiral environment. Free metal ions in solution are not detected by Vis-CD. This can be used to detect protein bound metals.
- **Near IR-CD** (750nm-950nm) can be used to investigate geometric and electronic structure by probing metal d-d transitions.

D

Protein Structure

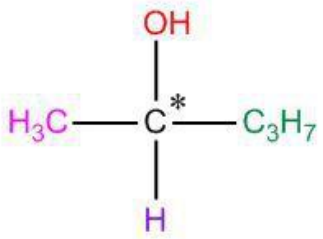
- Proteins possess a number of chromophores which can give rise to CD signals.
- In the far UV region (240-180 nm), which corresponds to peptide bond absorption, the CD spectrum can be analysed to give the content of regular secondary structural features such as a-helix and b-sheet.
- The CD spectrum in the near UV region (320-260 nm) reflects the environments of the aromatic amino acid side chains and thus gives information about the tertiary structure of the protein.
- CD has been used extensively to give useful information about protein structure, the extent and rate of structural changes and ligand binding. In the protein design field, CD is used to assess the structure and stability of the designed protein fragments.



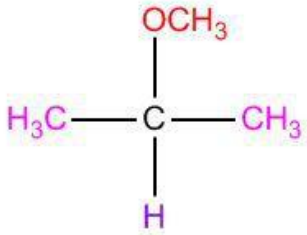


Amino Acid Structure

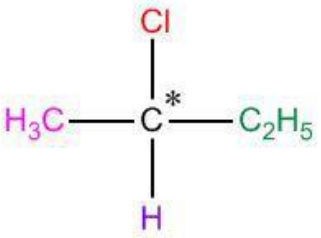
Kiralitas



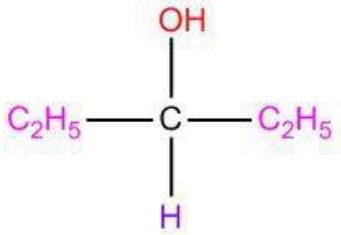
pentan-2-ol
atom C kiral



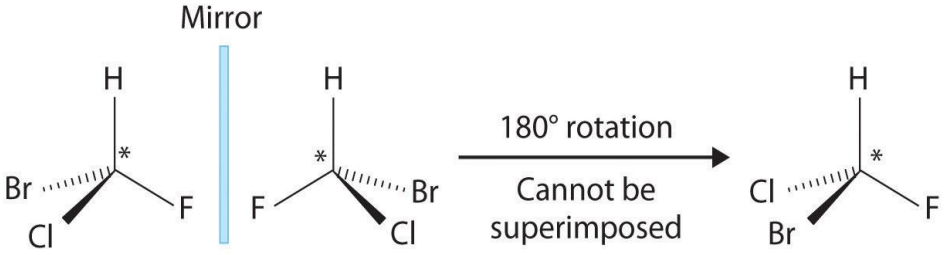
2-metoksipropana
atom C tidak kiral



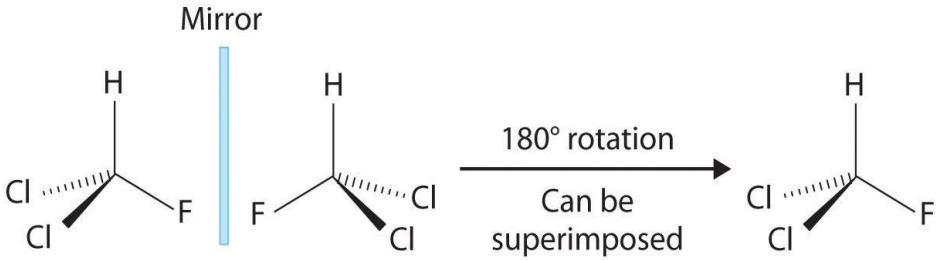
2-klorobutana
atom C kiral



pentan-3-ol
atom C tidak kiral



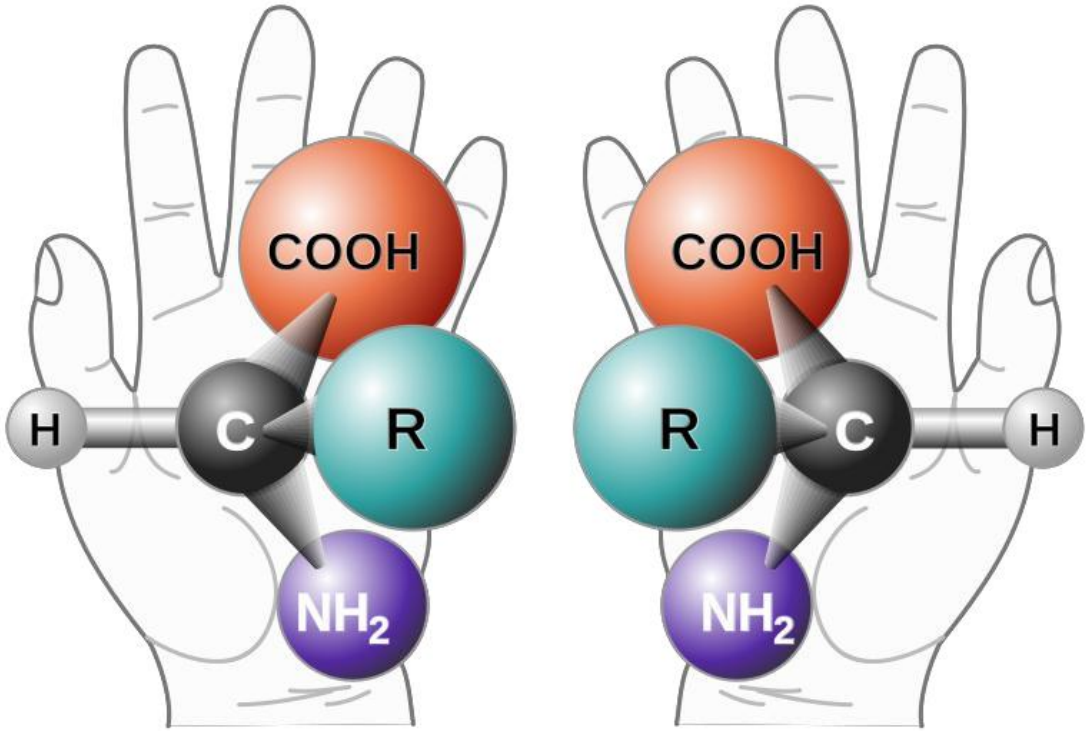
(a) Bromochlorofluoromethane



(b) Dichlorofluoromethane

- Molekul kiral adalah molekul yang memiliki bayangan cermin tidak superimposabel (tidak dapat bertumpukan)
- Kiralitas dapat muncul pada molekul organik (biasanya pada atom karbon) yang mengikat 4 gugus fungsi yang berbeda sehingga bentuknya asimetris

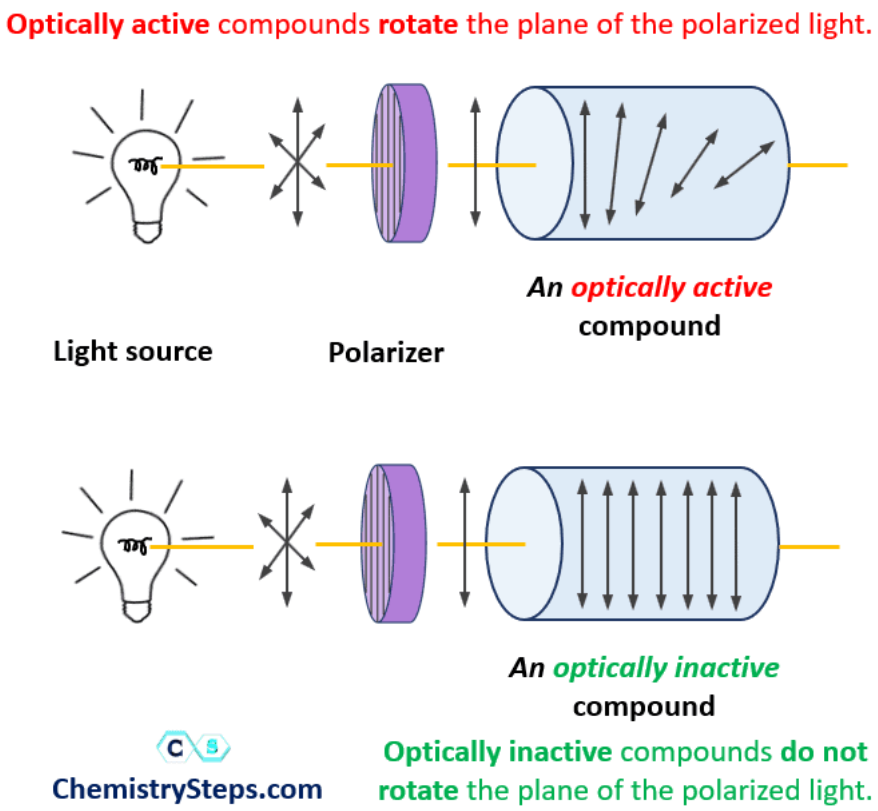
Perumpaan Kiralitas

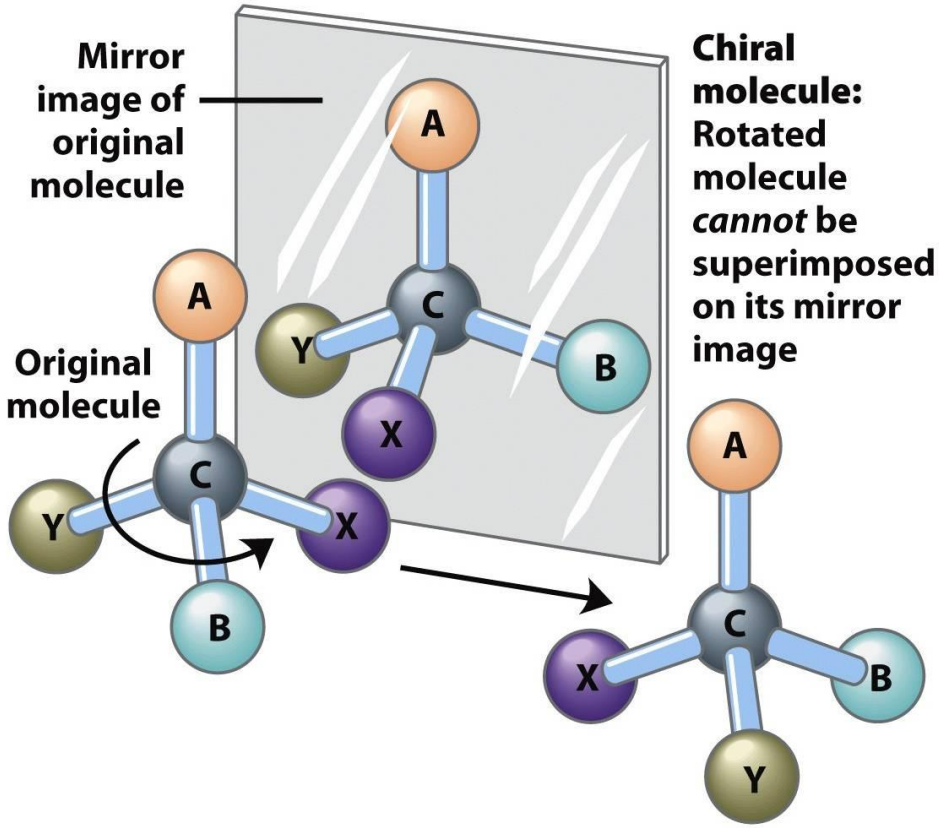


“Tangan manusia sebagai perumpamaan konsep kiralitas”

Tangan manusia diibaratkan seperti isomer. Namun secara optik jika tangan kanan dihadapkan ke cermin akan menghasilkan produk yg berbeda yaitu tangan kiri.

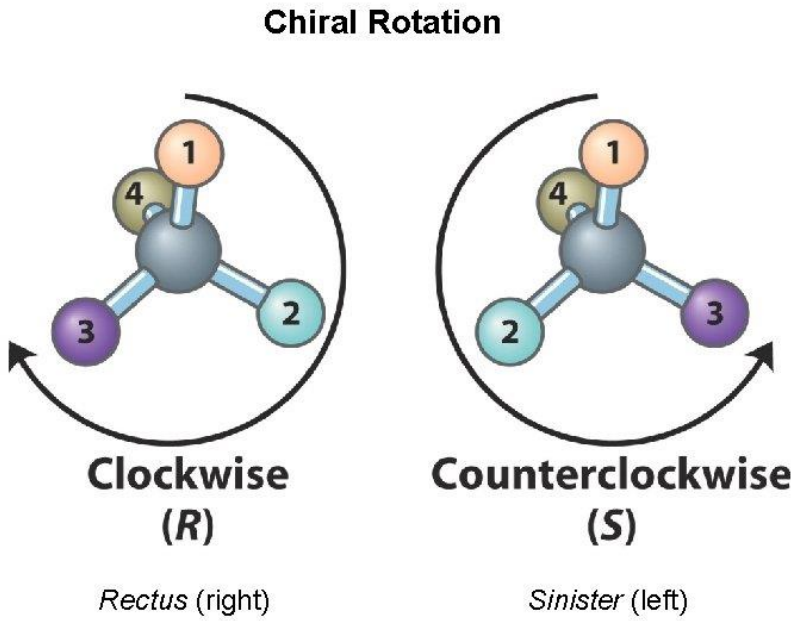
Optis Aktif

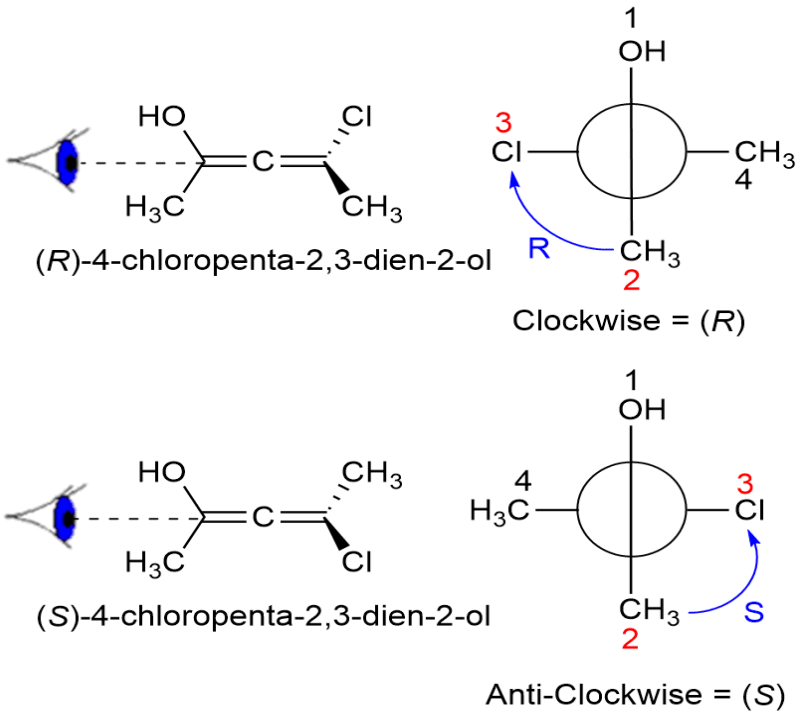




- Optis aktif adalah sifat suatu zat yang dapat memutar bidang cahaya terpolarisasi pada saat cahaya tersebut melewatinya.
- Kemampuan ini terjadi apabila molekul tersebut tidak simetris atau bersifat kiral. Sedangkan molekul yang simetris atau akiral adalah molekul yang tidak optis aktif, akibatnya molekul tersebut tidak mampu memutar bidang cahaya terpolarisasi.

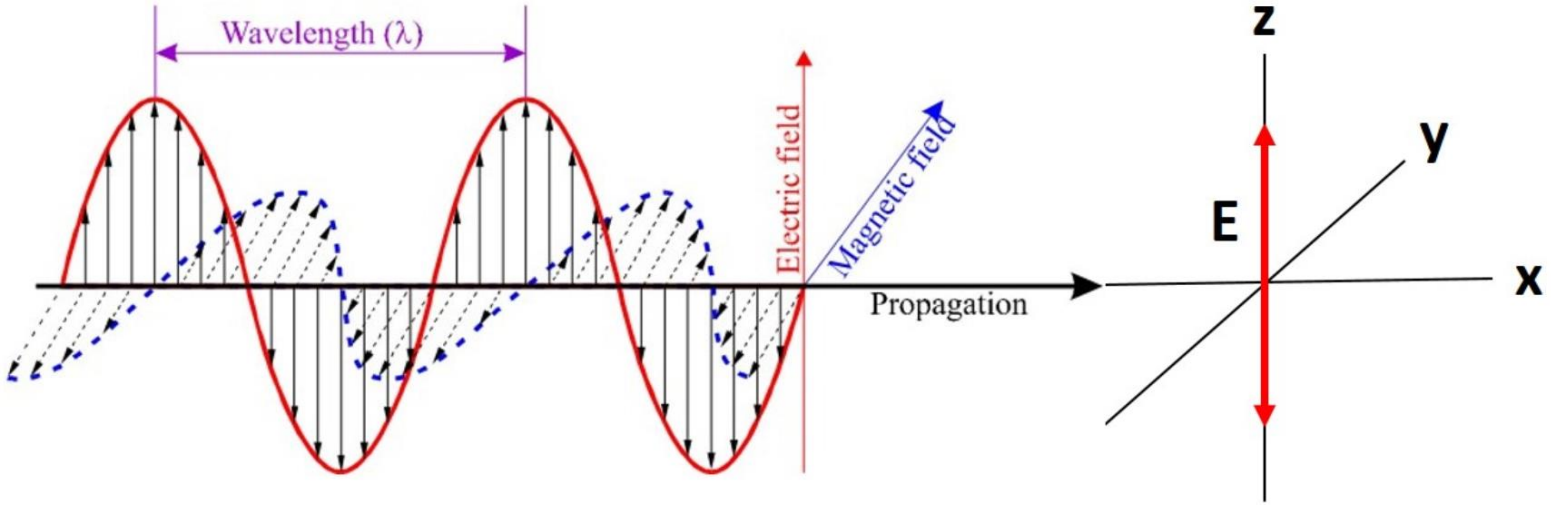
Arah Pemutaran Bidang Polarisasi

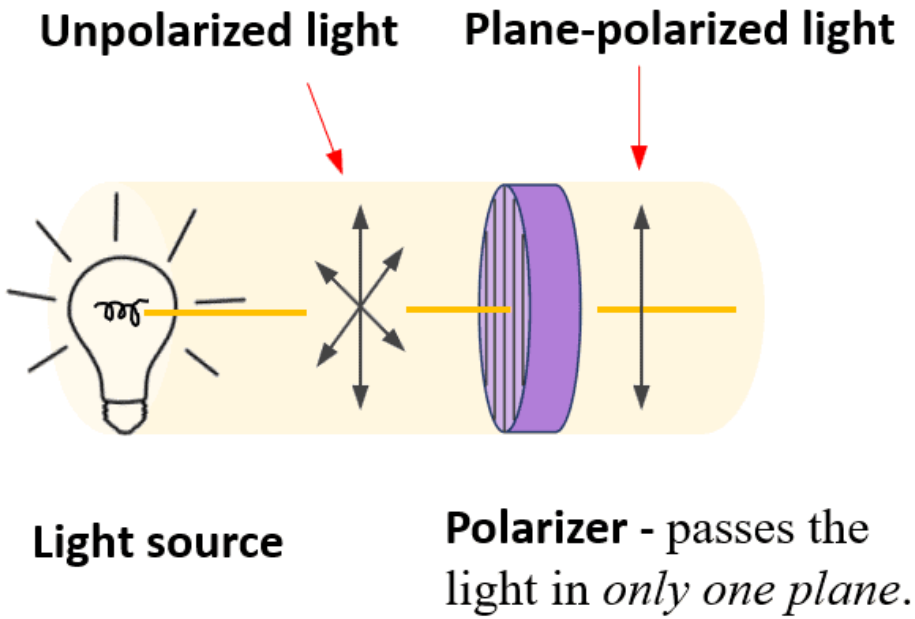




- Pemutaran bidang polarisasi dapat berupa dextro-rotary (+) bila arahnya searah dengan jarum jam, ataupun levo-rotary (-) bila arahnya berlawanan dengan arah jarum jam.
- Sistem R/S adalah sistem tatanama untuk menjelaskan enantiomer. Sistem ini berasal dari bahasa Latin:
  R (*Rectus)* = kanan S (*Sinister*) = kiri
- Suatu senyawa yang dapat sekaligus menjadi pemutar kanan dan kiri dinamakan zat rasemik.

Sinar Terpolarisasi





- Cahaya merupakan gelombang elektromagnetik yang terdiri dari getaran medan listrik dan getaran medan magnet yang saling tegak lurus.
- Apabila cahaya/sinar ini melalui suatu polarisator maka sinar yang diteruskan mempunyai getaran listrik yang terletak pada satu bidang saja dan dapat dikatakan sinar terpolarisasi bidang (linear).

Polarized of light

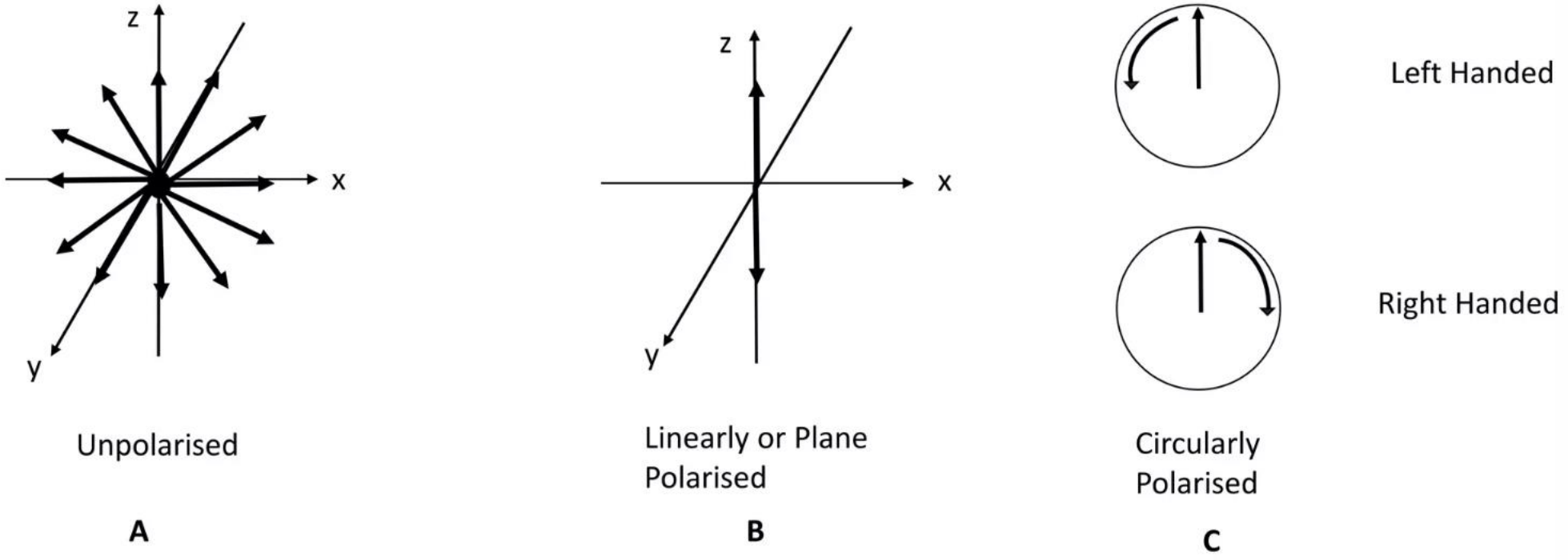
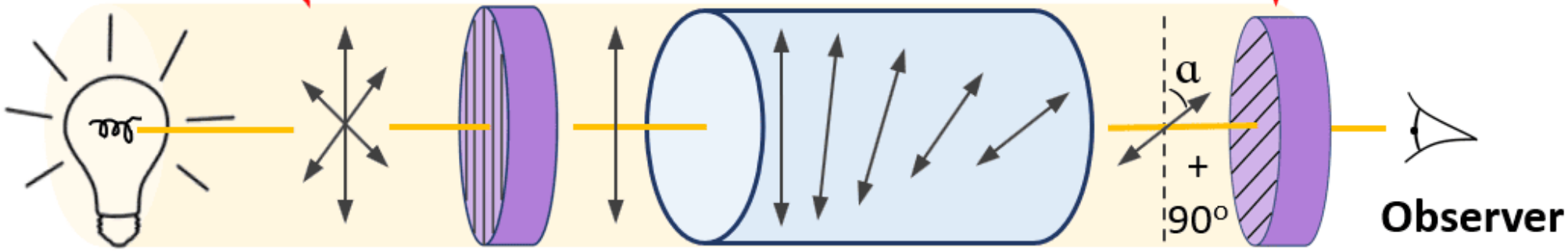


Figure 4: Schemes of the electric field components of unpolarized **(A)**, linearly or plane polarized light **(B)**. For circularly polarized light **(C).**



Light source

Polarizer

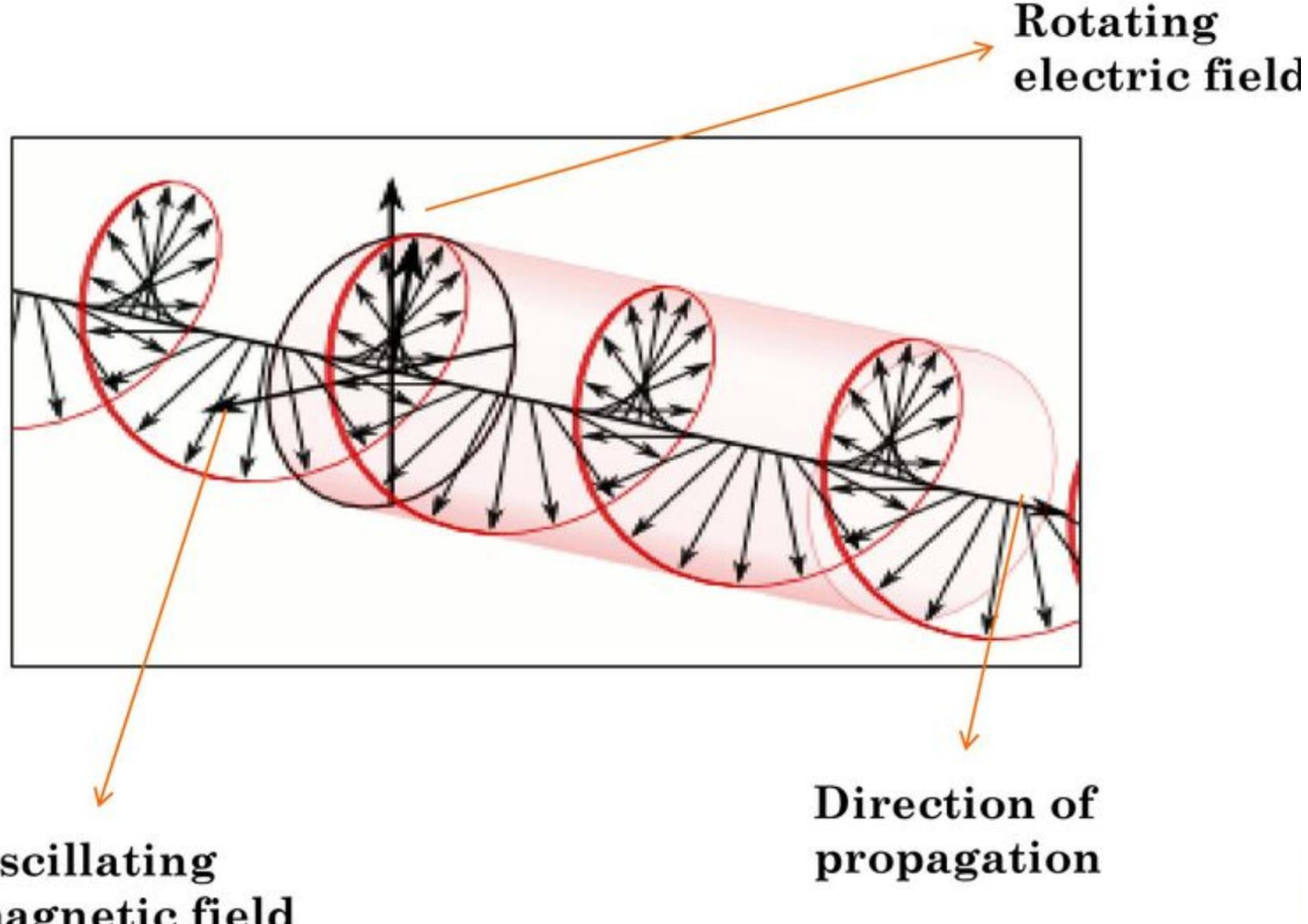
Polarimeter tube containing an ***optically active*** sample.

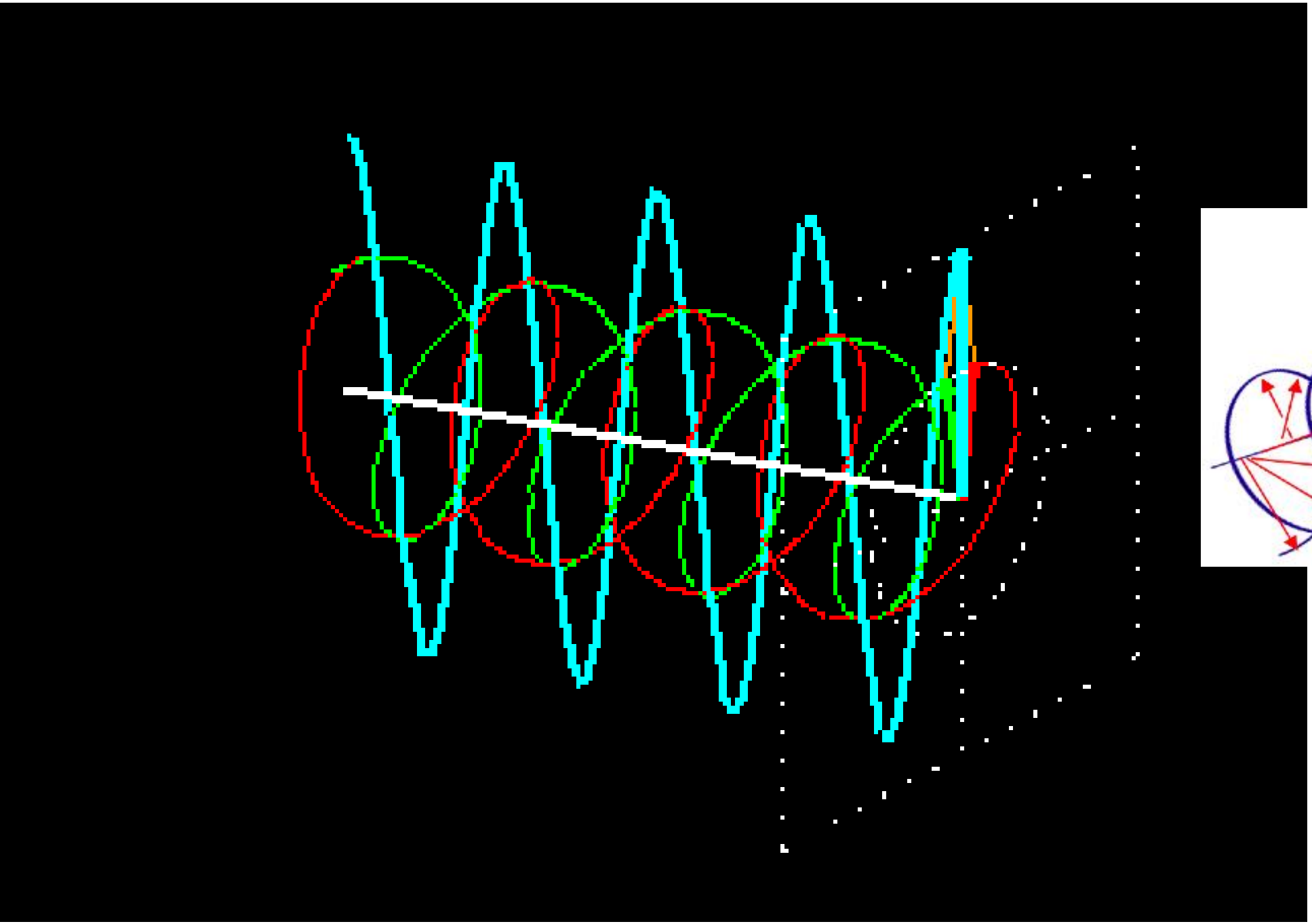
Analyzer

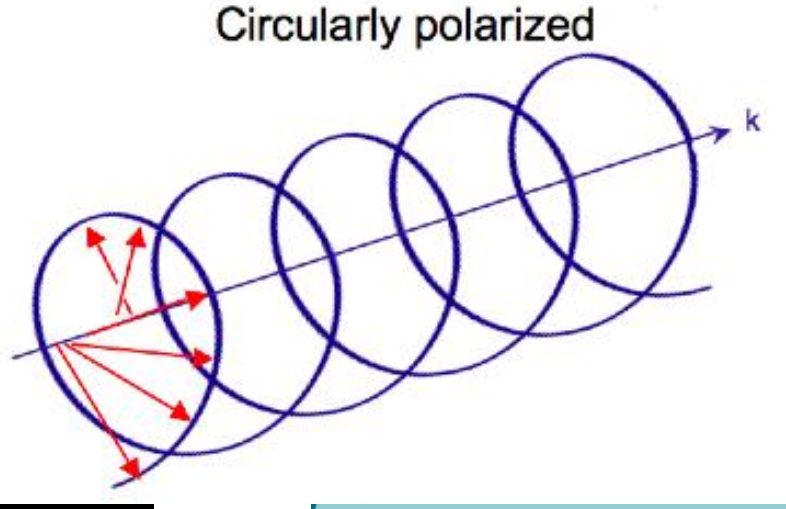
The polarizer passes the light in *only one plane*.

In this example, the plane of the light is rotated by 90°.
This is the ***observed rotation (α).***

CIRCULARLY POLARISED LIGHT






Circularly polarized
k

Circular Dichroism (CD)

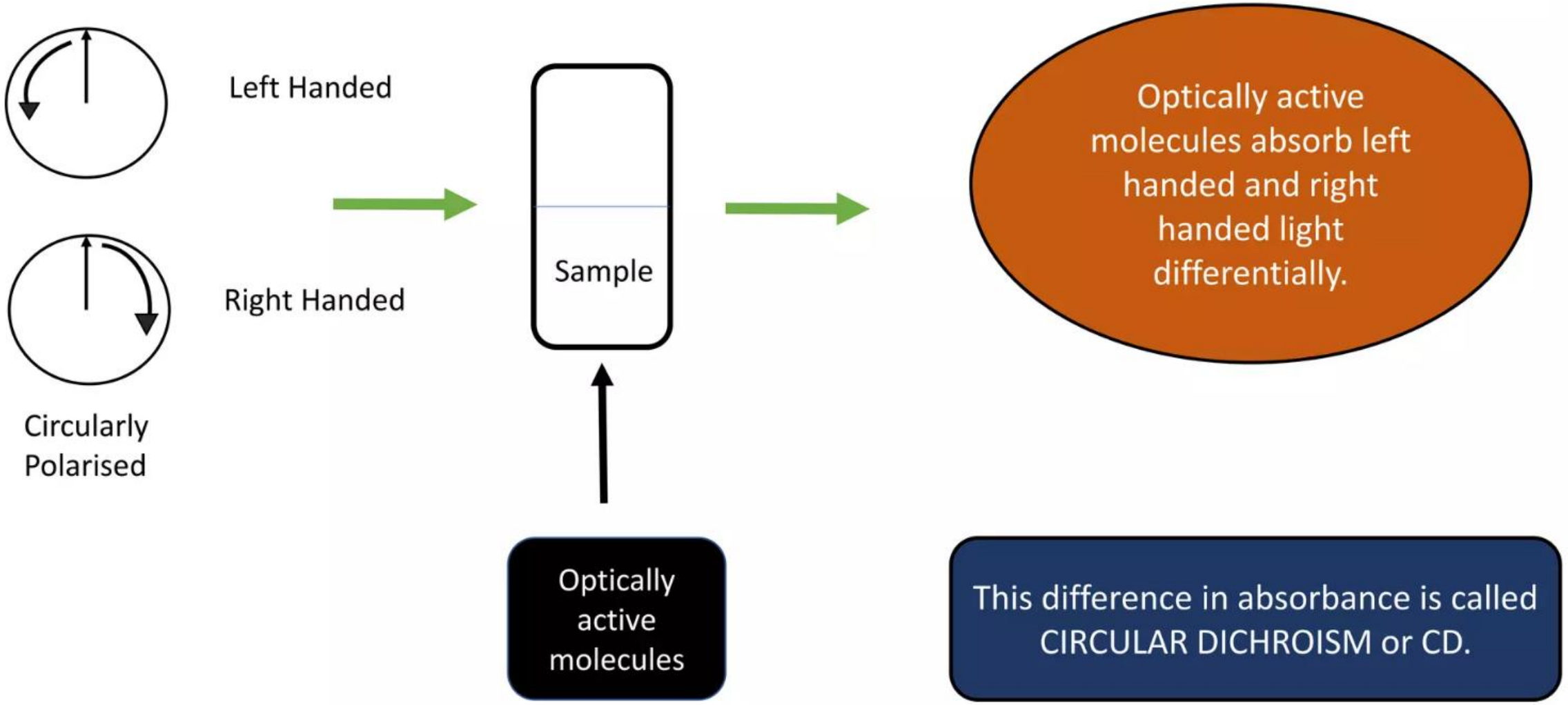


Figure 5: Schematic Representation of Circular Dichroism

Instrumentation For CD Spectropolarimeter

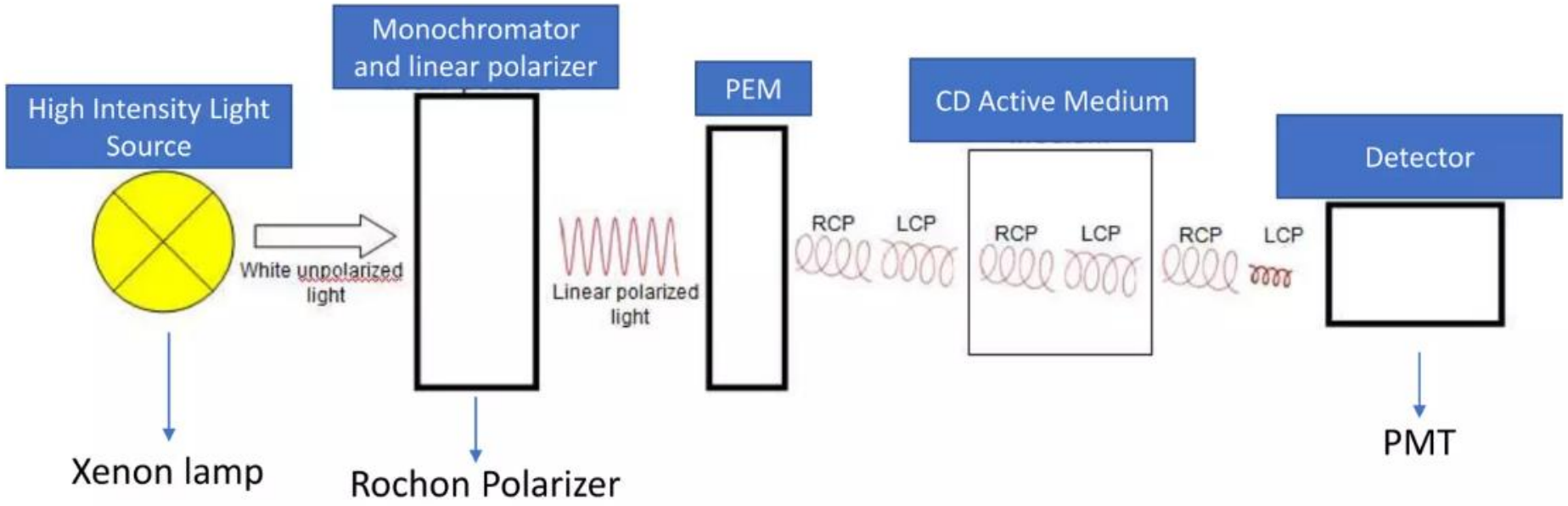
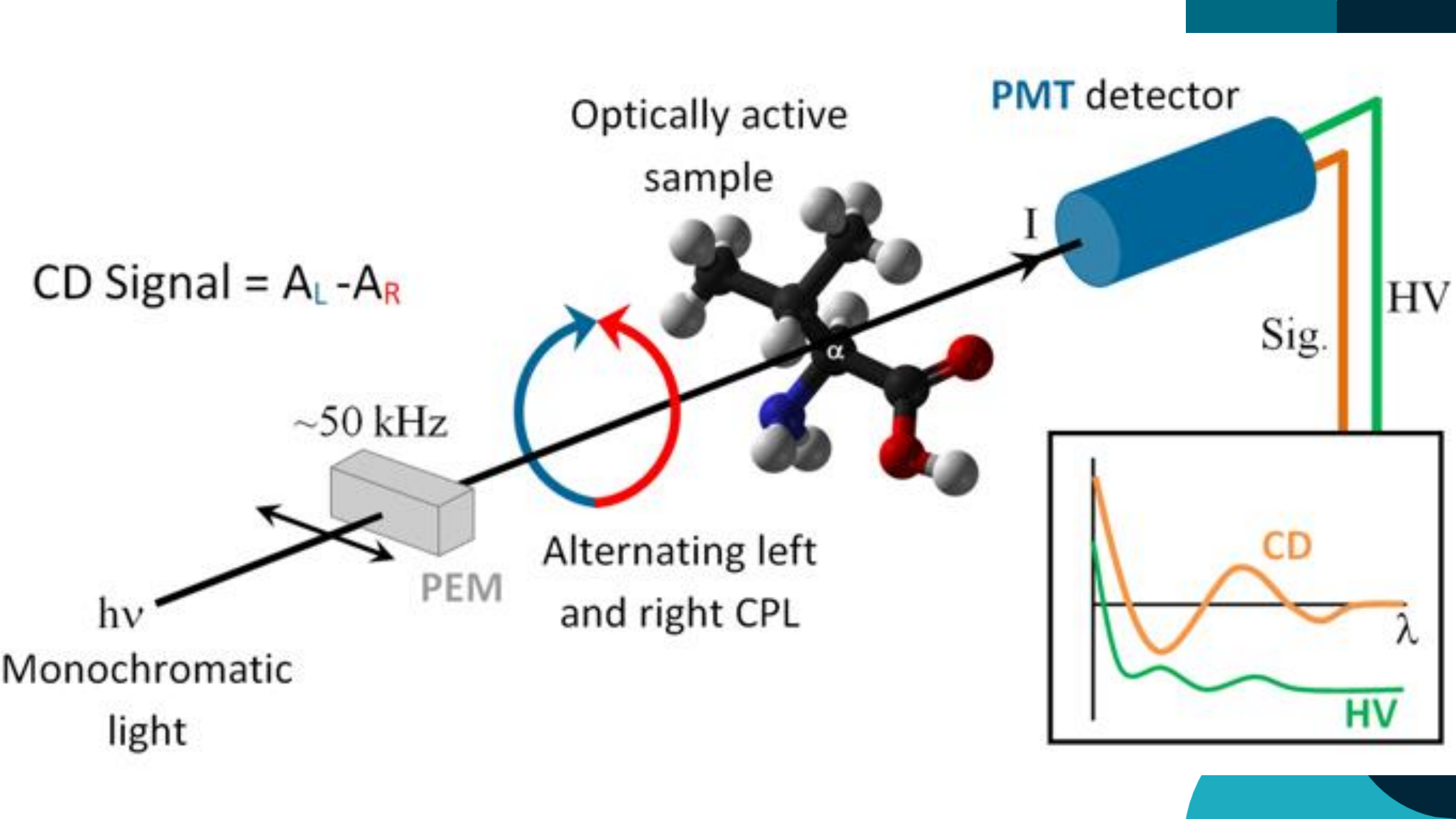


Figure 7: Instrumentation for a common CD spectropolarimeter

PEM = Photo Elastic Modulator
PMT = Photomultiplier Tube


PMT detector
Optically active sample
I
CD Signal = $A_L - A_R$
HV
Sig.
α
~50 kHz
PEM
Alternating left and right CPL
hν
Monochromatic light
CD
λ
HV

CD Spectrum

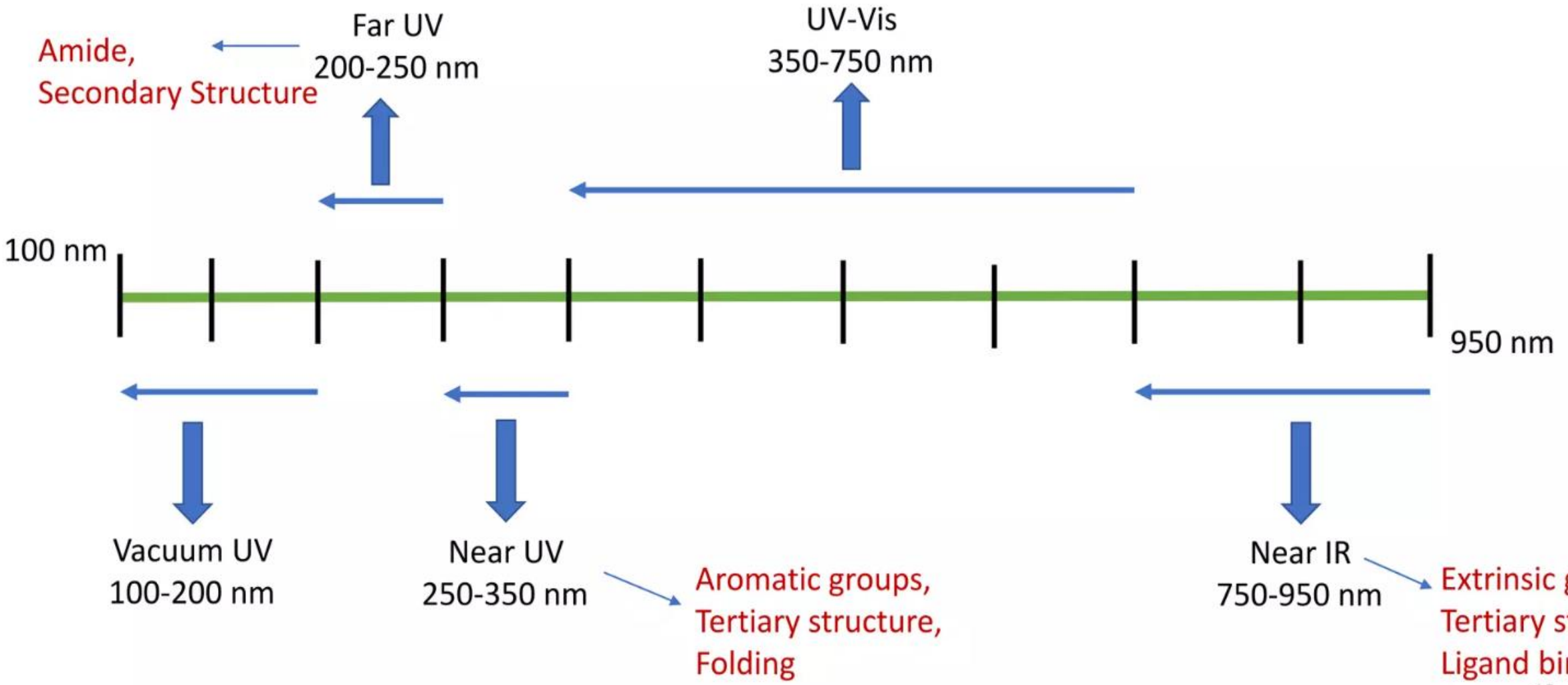


Figure 9: Schematic Representation of Classification of CD Spectrum Regions

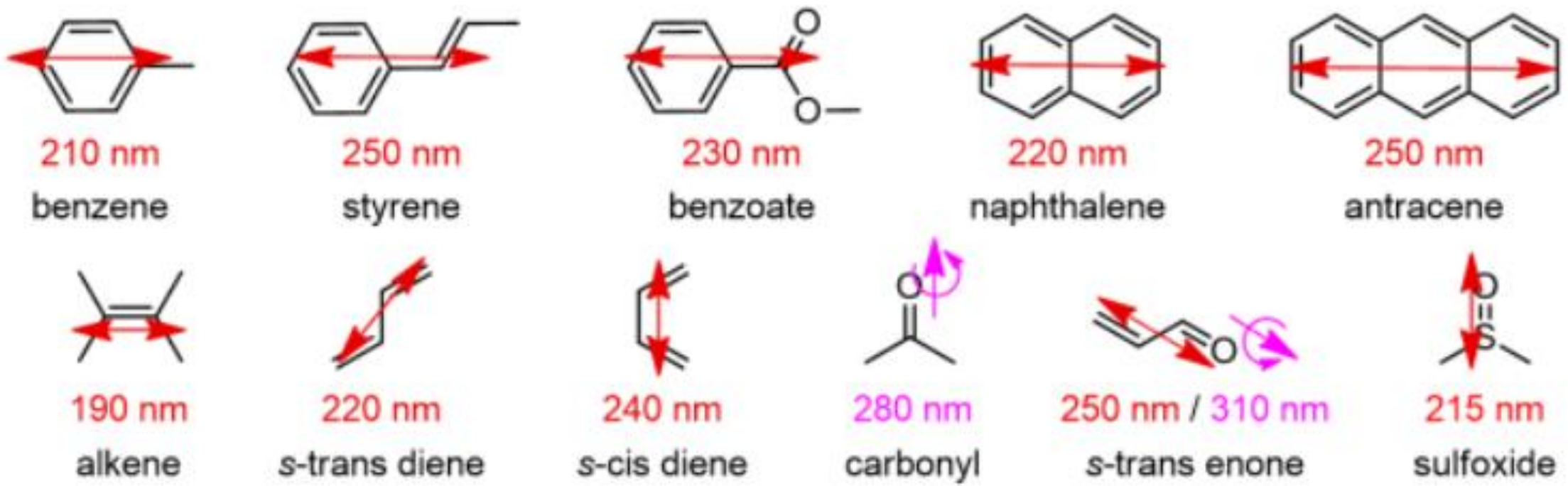


Figure 3. Common organic chromophores with their main UV electronic transitions; electric transition dipoles shown as double arrows, magnetic as single plus curved arrows.

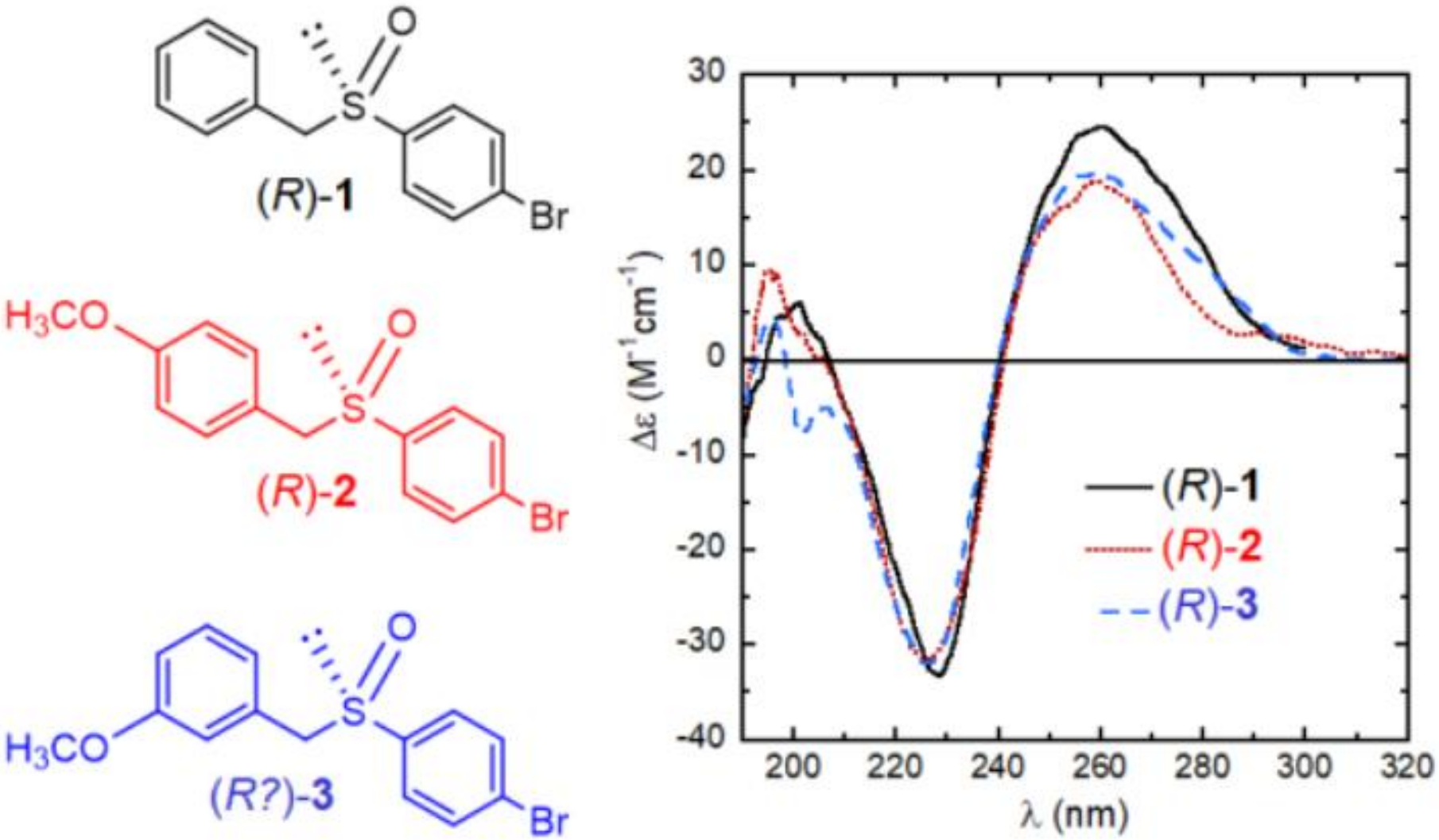


Figure 4. Structures and ECD spectra of sulfoxides (*R*)-**1**-**3**.

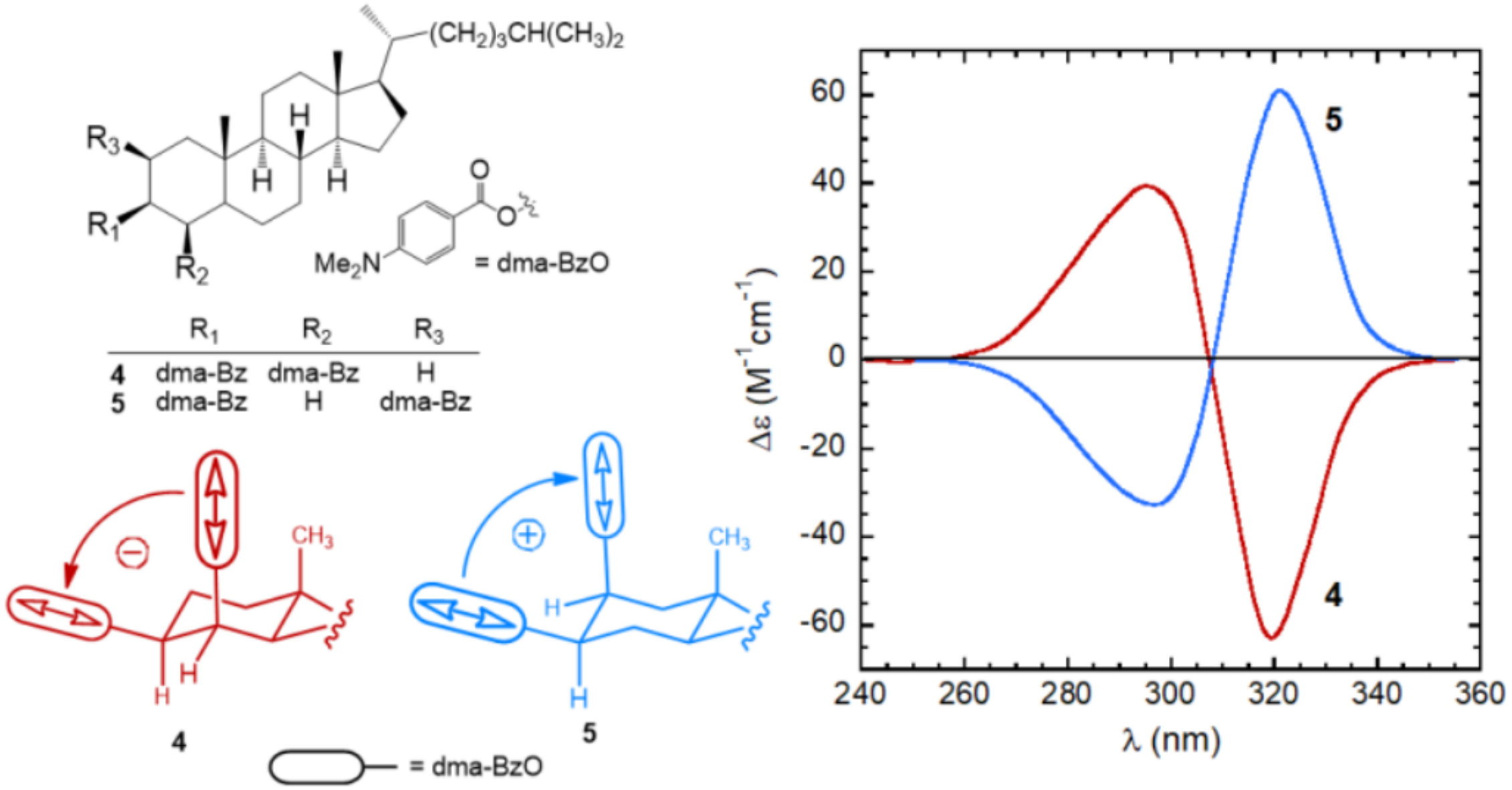
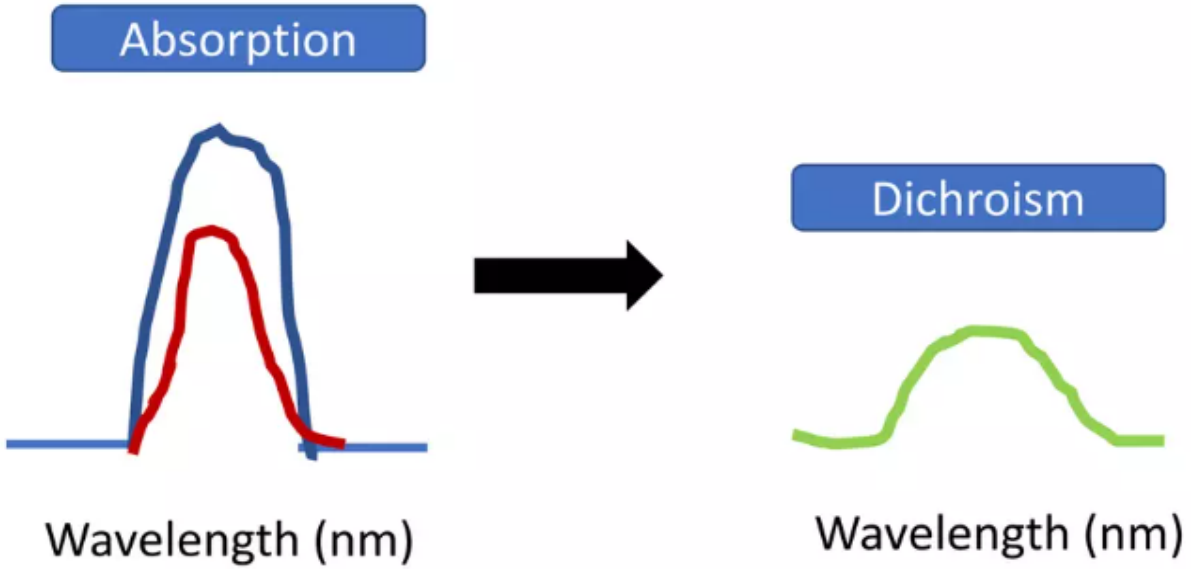


Figure 5. ECD spectra of *p*-dimethylaminobenzoates **4** and **5** from steroidal 1,2-diols,[8]

Measurement of Circular Dichroism

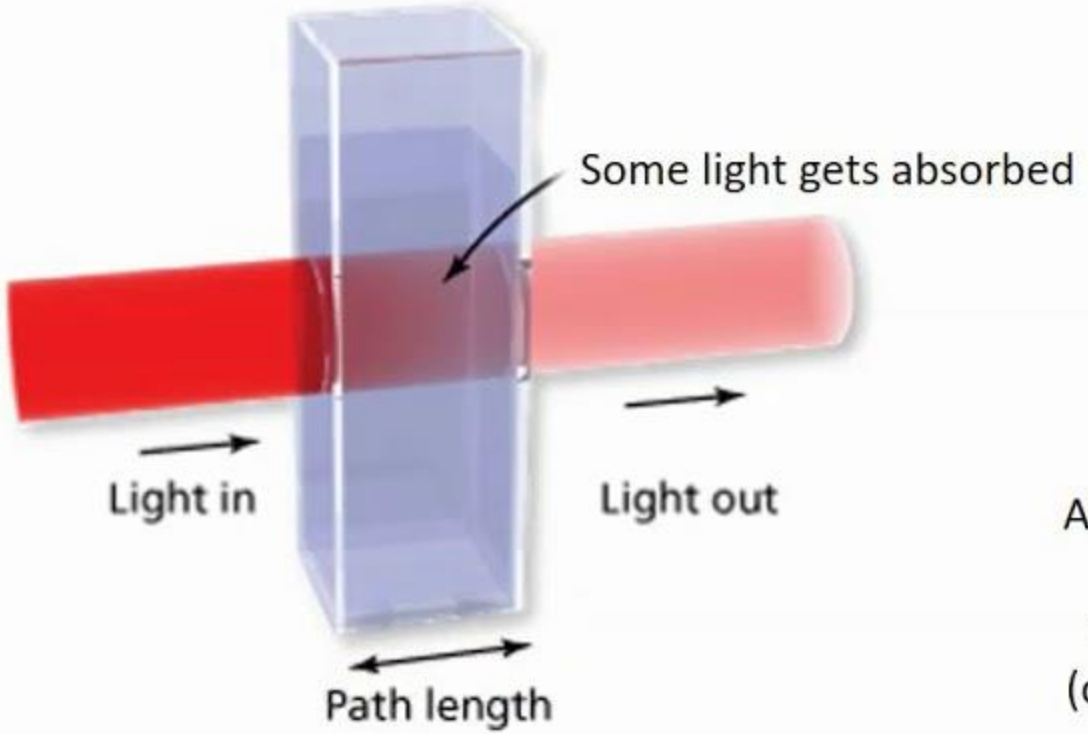


- **Circular Dichroism** = $\Delta A = A_L - A_R$
- $\Delta A = A_L - A_R$
- According to **Beer Lamberts Law**
- $A = \varepsilon cl$
- $\Delta A = \varepsilon_L cl - \varepsilon_R cl$
- $\Delta A = \Delta \varepsilon cl$
- $\Delta \varepsilon = \Delta A/cl$
- For unit concentration and unit length
- $\Delta \varepsilon = \Delta A$ = Circular Dichroism

Spectrophotometer

Pass Light Through the Solution

Know how much is sent in/measure how much comes out.



Beer-Lambert Law

A Constant

$$A = \varepsilon bc$$

Concentration of the solution (M)

Absorbance at a given wavelength (color) of light

Path Length: Distance light passes through the solution

CD Spectra of Protein Secondary Structures

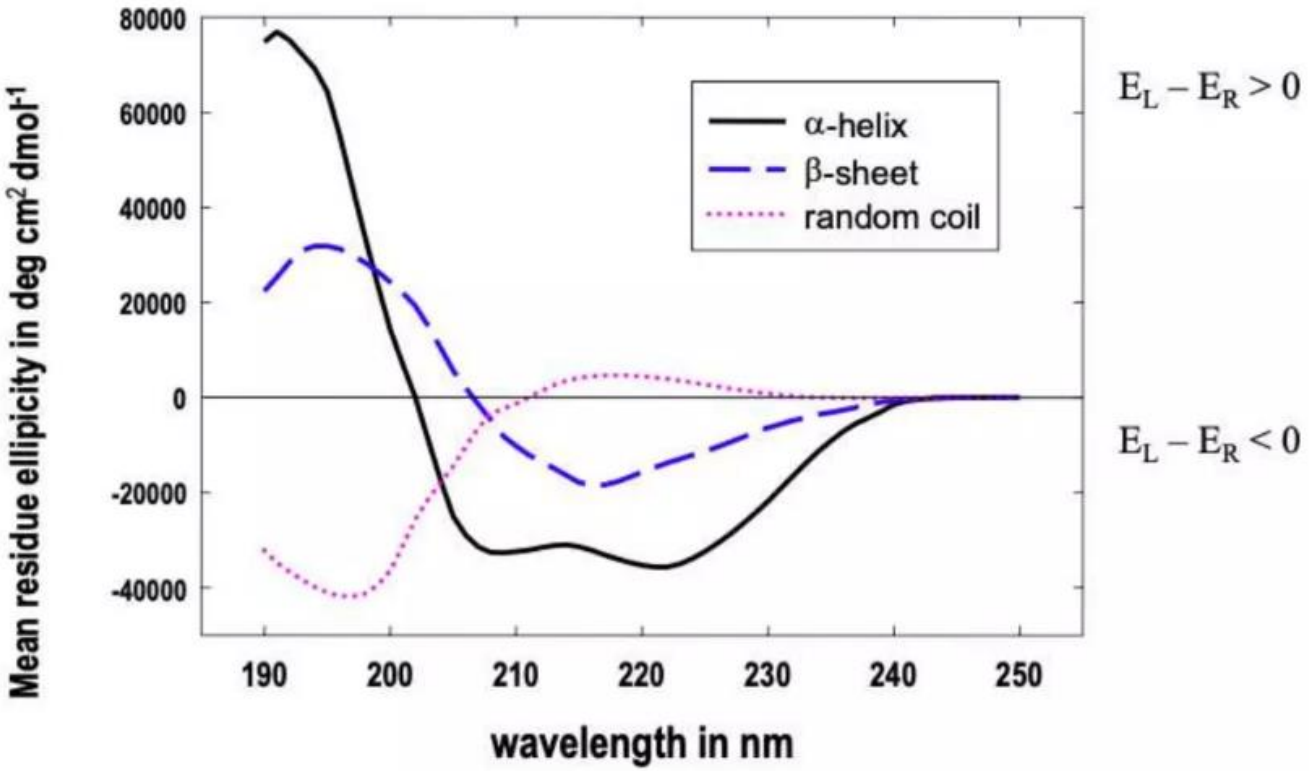


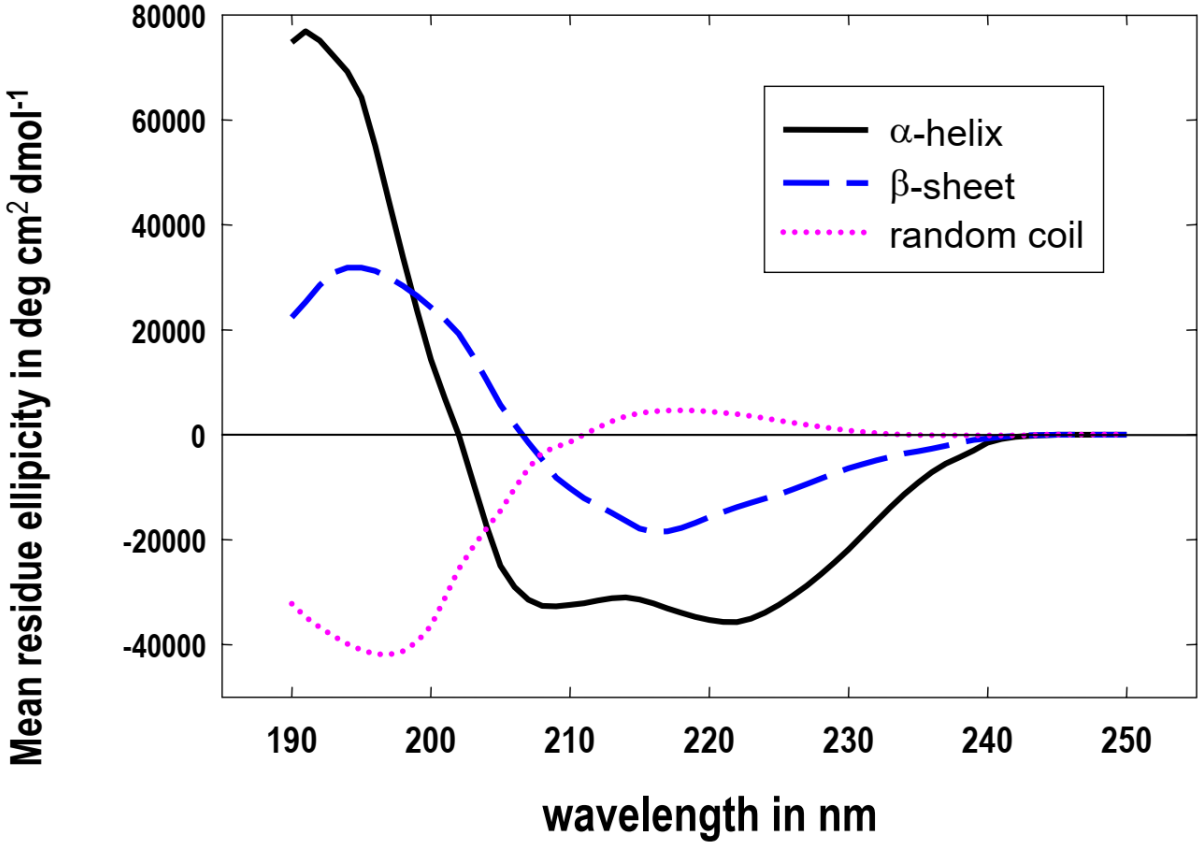
Figure 10: Fasman Standard Curve for Polylysine

	-ve band (nm)	+ve band (nm)
α-helix	222	192
	208	
β-sheet	216	195
Random coil	200	

Table 1: Features of CD Spectra of Protein Secondary Structures

Reference: Ranjbar B, Gill P. Circular Dichroism Techniques: Biomolecular and Nanostructural Analyses- A Review. Chemical Biology & Drug Design. 2009;74(2):101-120.

CD Signals for Different Secondary Structures



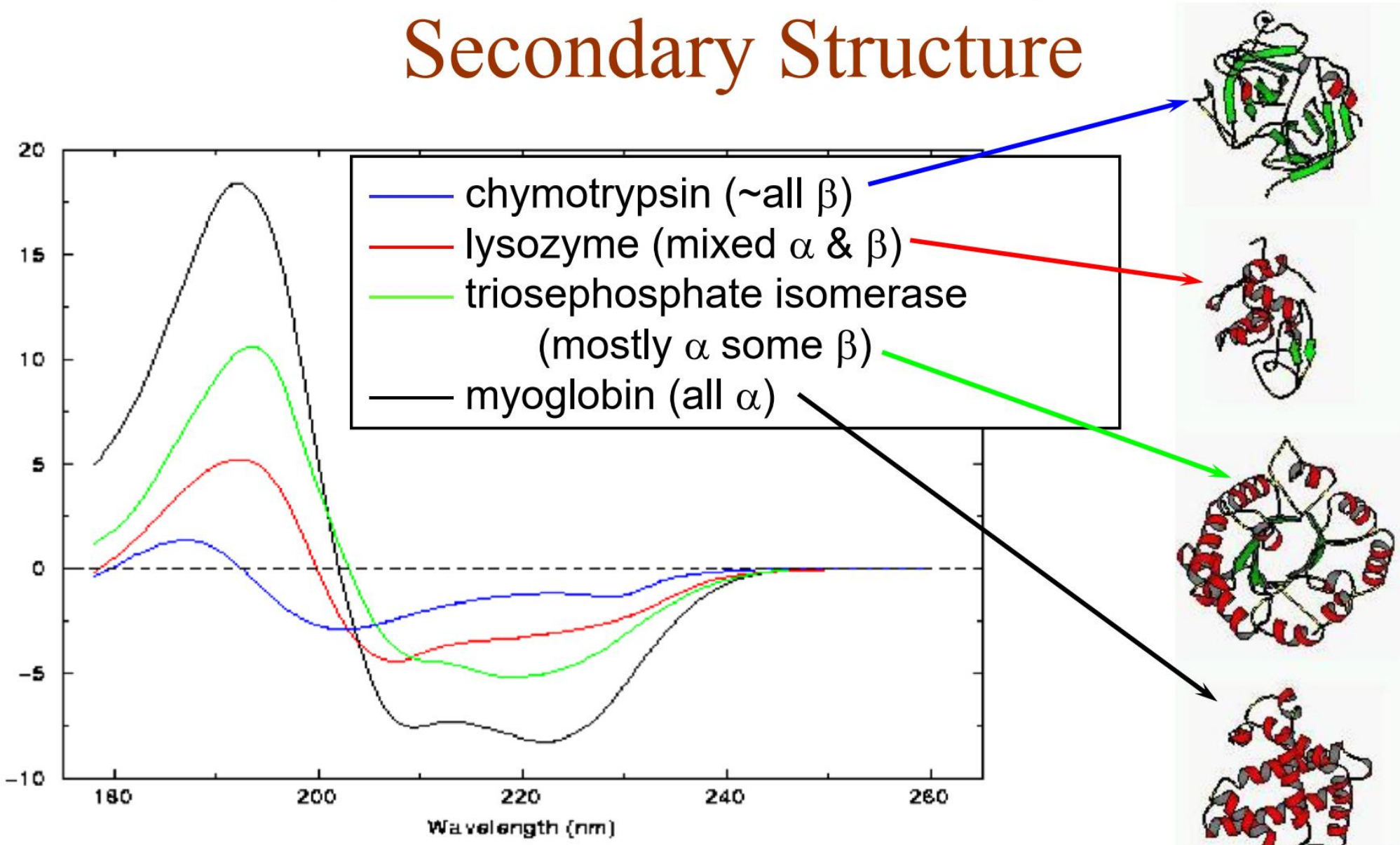
$E_L - E_R > 0$

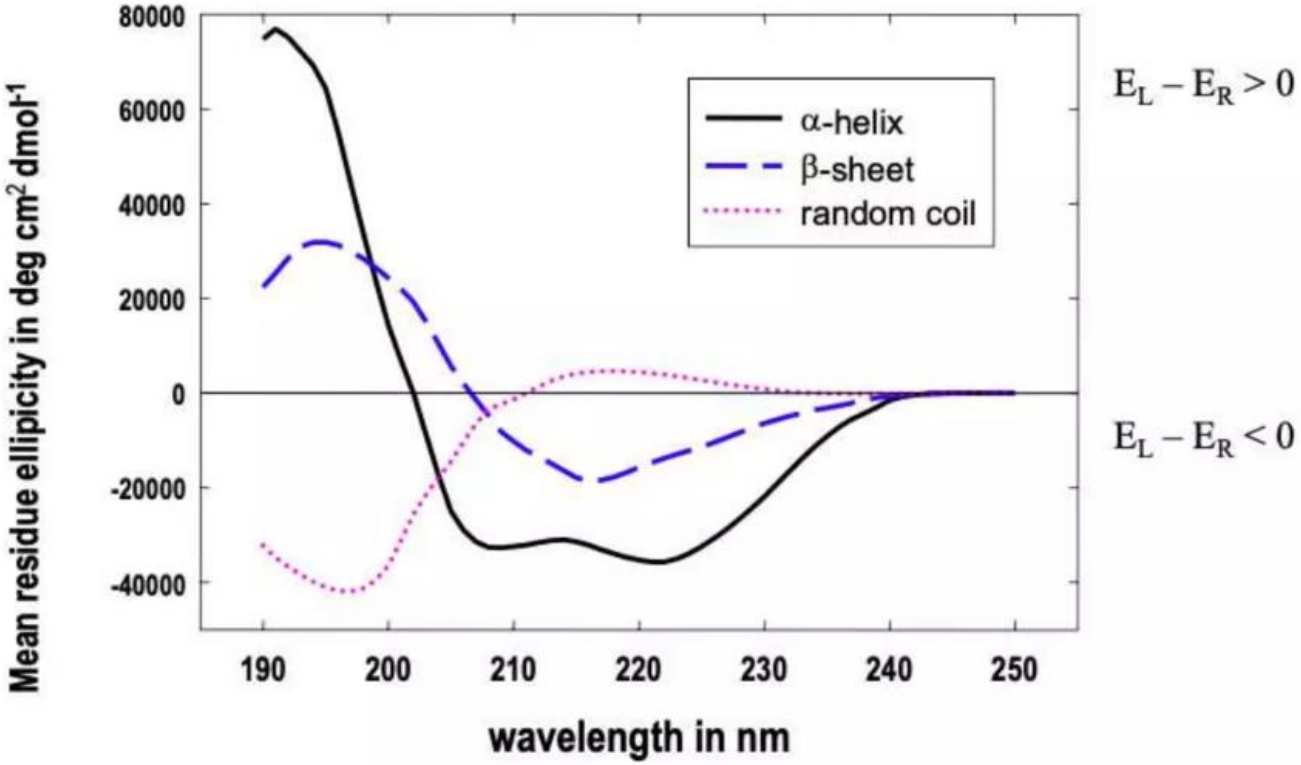
$E_L - E_R < 0$

- These are Fasman standard curves for polylysine in different environments

(data from ftp://jgiqc.llnl.gov)

Total Signal for a Protein Depends on its Secondary Structure





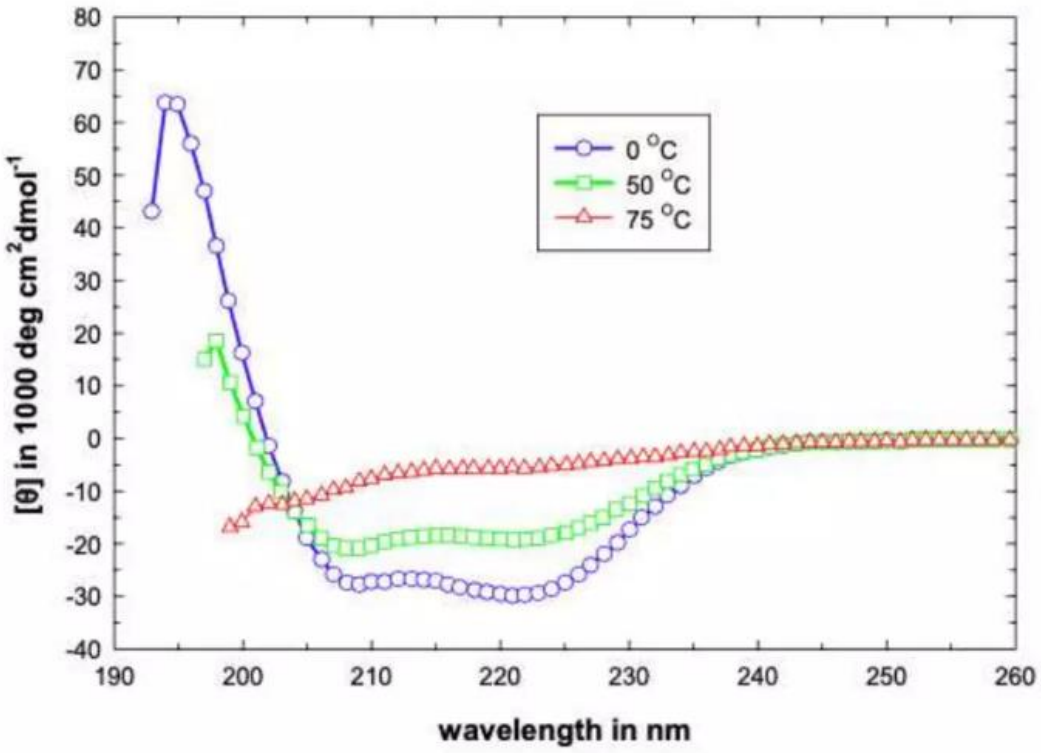
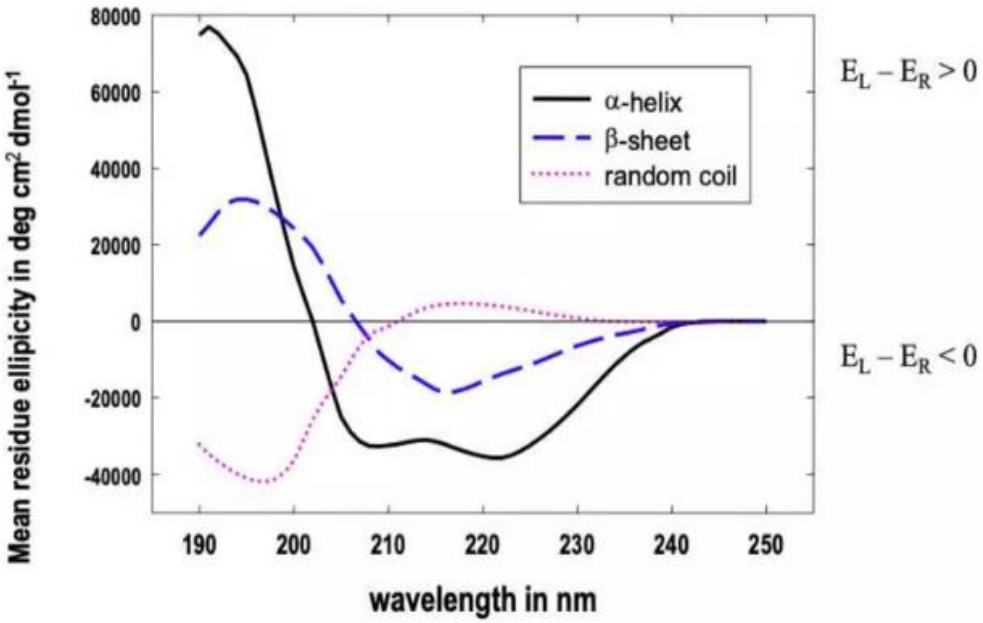


Figure 12: CD Signal of Protein Depending Upon Change in Temperature

Reference: Kelly S, Price N. The Use of Circular Dichroism in the Investigation of Protein Structure and Function. Current Protein & Peptide Science. 2000;1(4):349-384.



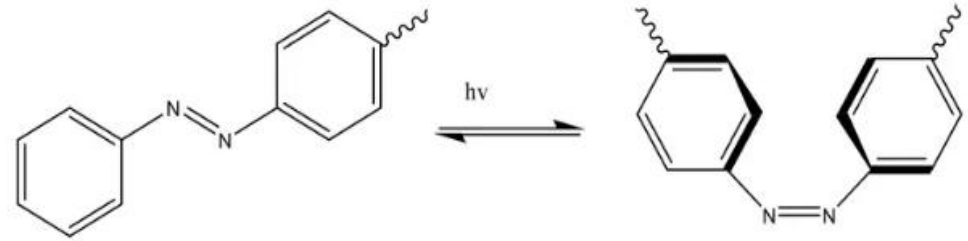
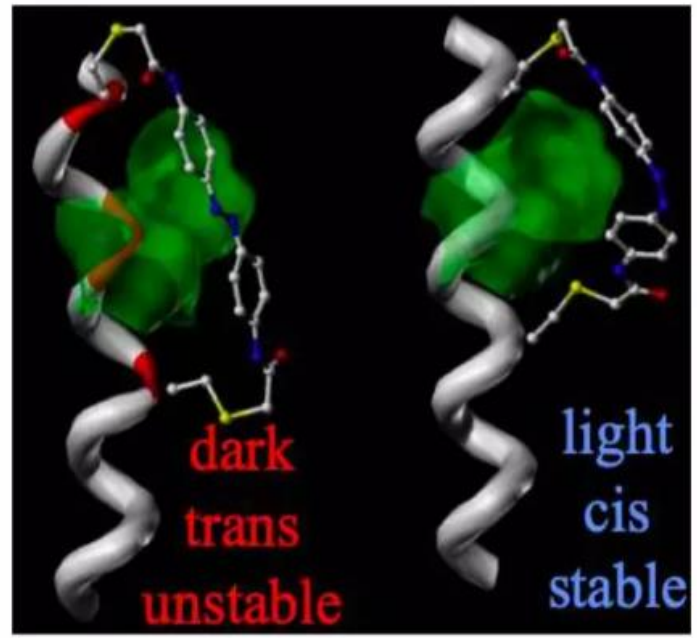


Figure 13: Chemical structure of the azobenzene cross-linker



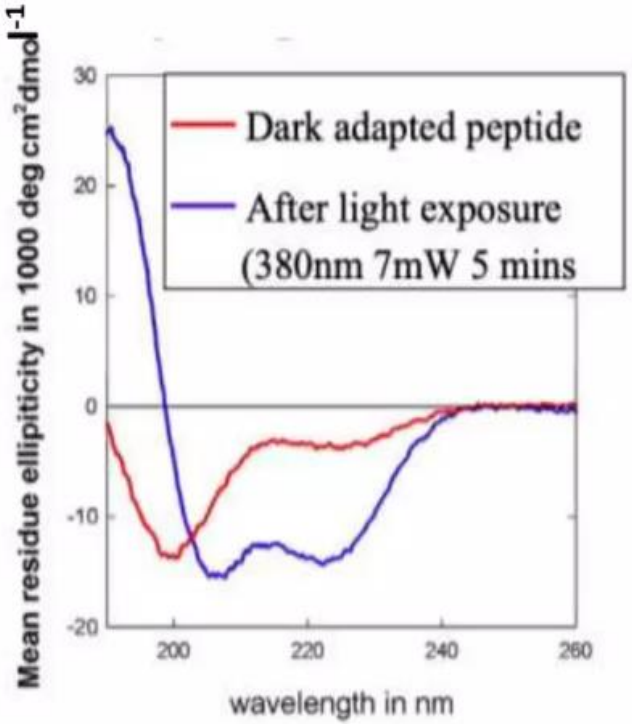


Figure 14: Models of the cross-linked peptide with the azobenzene group in the trans(left) and cis (right) conformations.

Reference: -Kumita J, Smart O, Woolley G. Photo-control of helix content in a short peptide. Proceedings of the National Academy of Sciences. 2000;97(8):3803-3808.

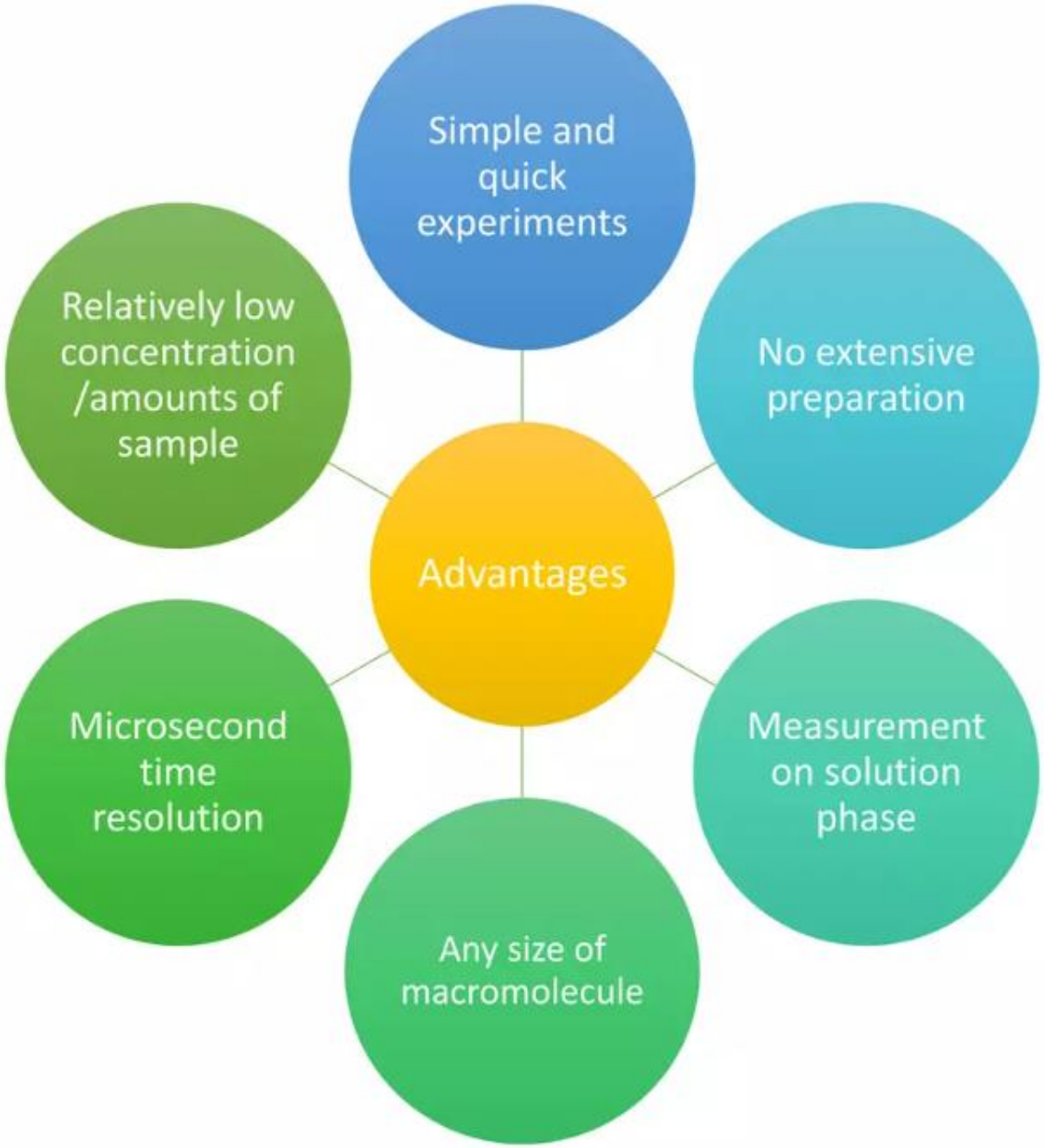


Figure 8: Advantages of CD Spectropolarimeter

DISADVANTAGE & COMPLICATIONS

- Carbohydrates can not be easily studied in CD.
- Aqueous buffer system used in CD often absorb in the range where structural feature exist.
- System should be completely devoid of O_2 to perform the experiment below 200nm.
- **Buffer selectivity:**

  Phosphate, Sulphate, Carbonate, Acetate buffer can not be used until they are prepared in the range of 10-40mM.
- **Solvent selectivity:**

  A large number of organic solvents like THF, $CHCl_3$, CH_2Cl_2 etc can not be used.
- **Lamp selectivity:**

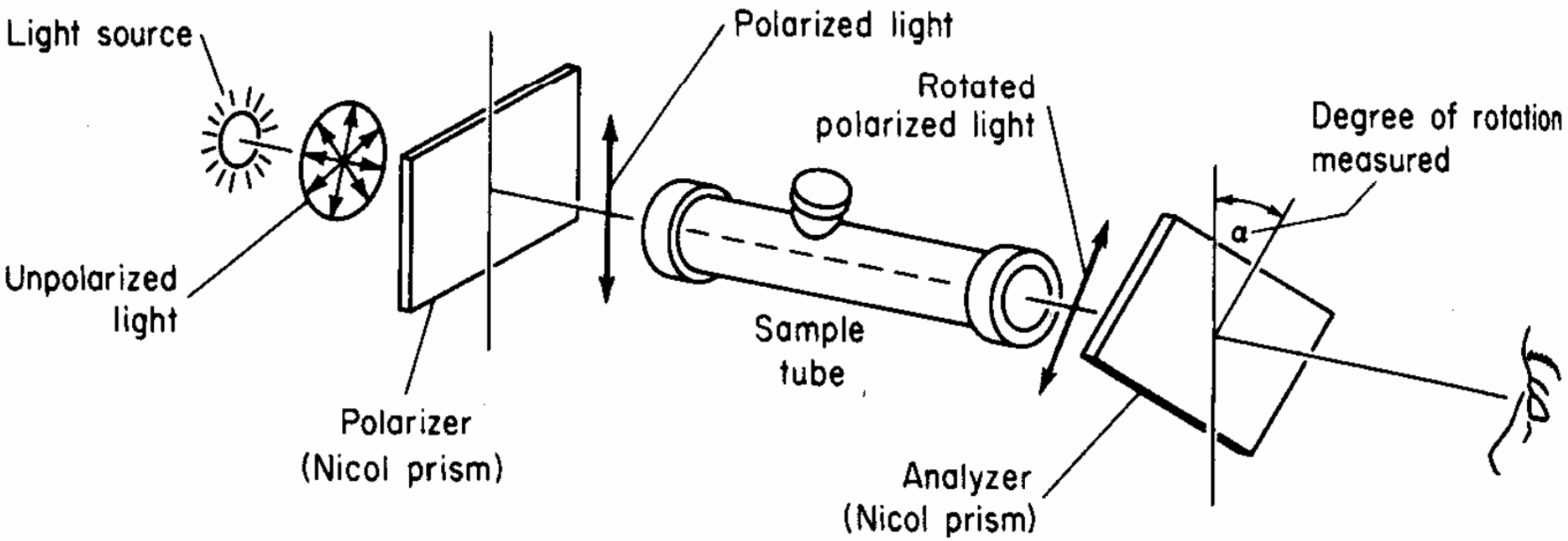
  Instead of ordinary Xe-arc lamps, use of high pressure short arc Xe lamps are essential for doing low UV-CD spectroscopy.

Circular Dichroism (CD) Spectropolarimeter

- CD is now routinely used to study biological macromolecules (i.e., proteins, nucleic acids, etc.) and also provides useful structural information.
- Recent advances have made it possible to improve the time resolution of natural and magnetically induced CD spectral measurements from the millisecond to nanosecond and picosecond time regimes.
- As an analytical instrument, the technology can determine changes in secondary structure in a qualitative and even semi-quantitative fashion.
- With the easy availability of a wide range of empirical algorithms for secondary structure calculations, new reference databases and other data analysis tools, CD-based techniques should prove to be even more valuable tools in structural biology over the next decades than CD has been in the past 40 or more years since it was first used to estimate biomolecular structures.

Polarimeter

Komponen Alat pada Polarimeter




POWER
P-2000
Polarimeter

Interference filter

A wide range of interference filters from UV-Vis to NIR

Light source switching mirror option

Light source

Up to two light sources can be installed. Available light sources are;

WI (Tungsten-Halogen lamp)

Na (Sodium lamp)

Hg (Mercury lamp)

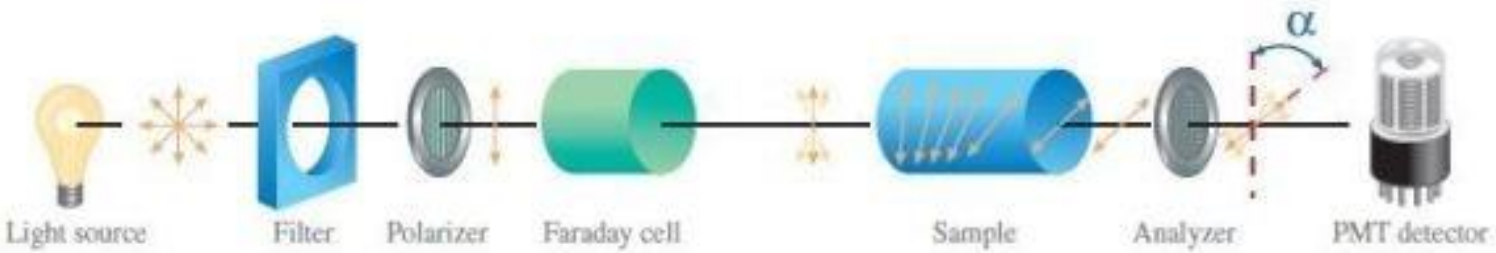
Polarizer

Faraday cells

Automatic accessory recognition

Analyzer

PMT detector

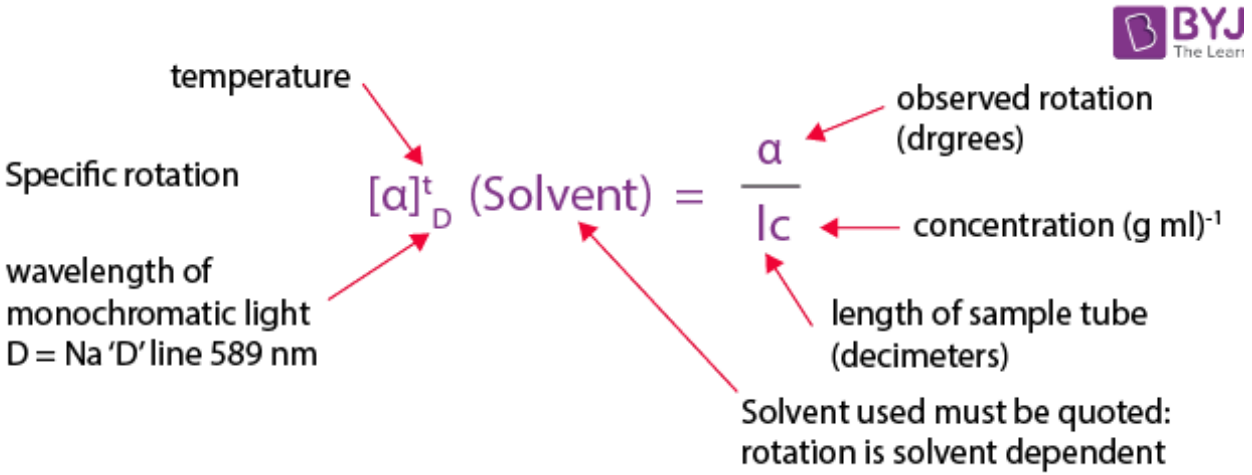


Penemuan Formula Kuantitatif Polarimetri

- Tahun 1812 Jean-Baptiste Biot mengamati bahwa besarnya rotasi optic tergantung pada panjang lempeng kuarsa yang digunakan dalam pengukuran. Biot juga memformulasikan rumus kuantitatif dari polarimetri.

Jean-Baptiste Biot

Hukum Biot dan Rotasi Optik



- Hukum Biot

$$\alpha = \pi l(n_L - n_R)/\lambda$$

L → panjang cahaya yang dilewatkan

n_L → indeks bias untuk cahaya terpolarisasi ke kiri

n_R → indeks bias untuk cahaya terpolarisasi ke kanan pada λ

$$\alpha \approx c$$
$$\alpha = kc$$
$$\alpha = [\alpha]_t dc$$
$$\alpha = rotasi\ optik$$
$$d = panjang\ tabung\ dalam\ dm$$
$$c = konsentrasi\ dalam\ g/mL$$
$$[\alpha]_t = rotasi\ spesifik$$

- Menurut hukum Biot: "besarnya rotasi optis dari suatu zat optis aktif akan sebanding dengan konsentrasi larutan dan tebal cairan"

- Sudut di mana bidang polarisasi diputar disebut rotasi optik. Besarnya rotasi optis yang diamati/diukur dari suatu larutan bergantung kepada jumlah senyawa dalam tabung sampel, panjang jalan/larutan yang dilalui cahaya, temperatur pengukuran, dan panjang gelombang cahaya yang digunakan.

- Rotasi spesifik suatu senyawa ($[\alpha]^{20}_D$) adalah besarnya sudut putar yang diakibatkan oleh 1,00 gram zat dalam 1,00 mL larutan yang barada dalam tabung dengan panjang 1,00 dm, pada temperatur dan panjang gelombang tertentu. Panjang gelombang yang lazim digunakan ialah 589,3 nm.

Faktor yang Mempengaruhi Rotasi Optik

- **Jenis zat**: Masing-masing zat memberikan sudut putaran yang berbeda terhadap bidang sinar terpolarisasi.
- **Panjang lajur larutan atau panjang tabung**: Jika lajur larutan diperbesar maka putarannya juga makin besar.
- **Suhu**: Makin tinggi suhu maka sudut putarannya makin kecil, hal ini disebabkan karena zat akan memuai dengan naiknya suhu sehingga zat yang berada dalam tabung akan berkurang.
- **Konsentrasi zat**: Konsentrasi sebanding dengan sudut putaran, jika konsentrasi dinaikkan maka putarannya semakin besar.
- **Jenis sinar (panjang gelombang)**: Pada panjang gelombang yang berbeda zat yang sama mempunyai nilai putaran yang berbeda
- **Pelarut**: Zat yang sama mempunyai nilai putaran yang berbeda dalam pelarut yang berbeda.

Contoh Soal

- Perkirakanlah besar dan arah dari rotasi sinar bila larutan maltose dengan konsentrasi 0,2 gram/ml diukur dalam kuvet 20 cm dengan menggunakan lampu Na pada suhu 20°C
- Jawab:
- $\alpha = [\alpha]^{20}_{D} dc = 130,5 \times 2 \times 0,2 = 52,2°$
- Larutan menyebabkan pemutaran cahaya 52,2° kearah kanan

Rotasi Spesifik Beberapa Senyawa Optis Aktif

Chemical name	Specific rotation $[\alpha]_D$
D-glucose	+52.7
Lactose	+55.4
D-fructose	-92.4
L-arabinose	+104.5
D-mannose	+14.2
D-arabinose	-104.5
D-xylose	+18.8
D-galactose	+80.2
Sucrose	+66.5
Maltose	+130.5
Dextrin	+195

Contoh Soal

- Hitunglah rotasi spesifik dari sampel murni 2-oktanol yang memberikan rotasi yang diamati +14.8° pada tabung sampel dengan panjang 20 cm dengan konsentrasi 2-oktanol adalah 0.838 gram/mL.
- Jawab: $[\alpha]^{20}_{D} = \frac{\alpha}{l.c} = \frac{14.8}{0.2\ x0.838} = +88.3°$

Contoh Soal

- Hitunglah konsentrasi dari D-glukosa yang memberikan rotasi yang diamati +20.5° pada tabung sampel dengan panjang 20 cm yang memiliki rotasi spesifik dengan menggunakan lampu Natrium pada suhu 20°C sebesar +52.7°

- Jawab: $[\alpha]^{20}_{D} = \frac{\alpha}{l.c}$

$$c = \frac{\alpha}{[\alpha]\ 20_D \times l}$$

$$c = \frac{20.5}{52.7 \times 0.2}$$

$$c = 1.944 \text{ gram/ml}$$

Aplikasi Polarimetri

- Mengukur rasio enantiomer dalam larutan.
- Memantau laju reaksi isomerisasi.
- Mengidentifikasi isomer yang ada dalam sampel. Isomernya adalah dekstro-rotasi jika ia memutar cahaya terpolarisasi searah jarum jam dan isomernya adalah rotasi Levo jika ia memutar cahaya terpolarisasi dalam arah berlawanan arah jarum jam.
- Mengidentifikasi sampel yang tidak diketahui berdasarkan rotasi spesifiknya. Data rotasi spesifik juga digunakan untuk menghitung konsentrasi dan kemurnian sampel tersebut.

Industri makanan

- Polarimetri digunakan dalam industri makanan untuk mengontrol kualitas produk asli, produk antara dan akhir, penentuan konsentrasi, dan kontrol kemurnian.
- Aplikasi pada industri gula: gula (sukrosa, levulosa, glukosa, dll.), sirup gula, pati, pemanis bebas gulaseperti isomalt, dll.
- Aplikasi pada industri susu: laktosa, sukrosa, laktoglobulin, asam laktat, ester, dll.
- Aplikasi pada industri anggur: analisis gula pada pokok anggur, asam tartarat, ester, dll.
- Aplikasi pada industri buah: analisis gula dalam sirup buah (levulosa), asam dan ester (asam malat, dll.), minyak atsiri, dll.

Industri farmasi

- Polarimetri digunakan dalam industri farmasi untuk mengontrol kemurnian dan penentuan konsentrasi zat sesuai dengan persyaratan Farmakope dengan pengukuran spesifik dan rotasi optik.
- Aplikasi pada penentuan alkaloid: kokain, kodein, nikotin, morfin sulfat, dll.
- Aplikasi pada penentuan asam amino: asparagin, asam glutamat, dll.
- Aplikasi pada penentuan senyawa organik: asam askorbat, mentol, kamper, dll. Lainnya: steroid, antibiotik, serum,vitamin, dll.

Obat

Polarimetri digunakan dalam aplikasi:

- Penelitian terkait gula dan albumin dalam urin
- Penelitian terkait hormon
- Penelitian terkait enzimologi dan toksikologi

Industri kosmetik

- Polarimetri digunakan untuk mengontrol kemurnian dan identifikasi minyak esensial dan esensi optik aktif seperti minyak lemon, minyak jeruk, minyak lavender, dll.

Industri kimia

- Polarimetri digunakan untuk mengontrol kemurnian dan pengukuran konsentrasi, identifikasi, dan karakterisasi senyawa, seperti:
- cairan organik
- Biopolimer
- Polimer sintetis
- Polimer organik

Penelitian

- Polarimetri digunakan untuk analisis analisis struktur senyawa optis aktif
- Perbedaan isomer optik

ISSN 1990-7931, Russian Journal of Physical Chemistry B, 2015, Vol. 9, No. 6, pp. 890–894. © Pleiades Publishing, Ltd., 2015.

KINETICS AND MECHANISM
OF CHEMICAL REACTIONS. CATALYSIS

Polarimetric Study of Rate Constant for Mutarotation of Chiral Levofloxacin, Ofloxacin, Sparfloxacin and Ciprofloxacin Drugs[1]

S. D. Bajaj, P. V. Tekade, and Manisha Chirde

Department of Chemistry, Jankidevi Bajaj College of Science, Jamanalal Bajaj Marg, Civil Lines, Wardha
e-mail: pradiptekade@gmail.com

Received November 26, 2014

Abstract—The polarimetric study of levofloxacin, ofloxacin, Sparfloxacin and Ciprofloxacin drugs has been carried out. Specific rotation and rate constant of mutarotation has been calculated with respect to time. It was found that the specific rotation and rate constant increases with increase in time. The specific rotation of ofloxacin and ciprofloxacin was found to be greater than that of sparfloxacin and levofloxacin. On the other hand the rate constant of mutarotation for sparfloxacin and ciprofloxacin was found to be greater than that of levofloxacin and ofloxacin.

Keywords: polarimetric study, chiral drugs, specific rotation, rate constant, mutarotation

DOI: 10.1134/S1990793115060214

Anal Chem. 2021 Apr 6;93(13):5403-5411. doi: 10.1021/acs.analchem.0c04651. Epub 2021 Mar 26.

Continuous-Wave Cavity-Enhanced Polarimetry for Optical Rotation Measurement of Chiral Molecules

Dang-Bao-An Tran [1], Katherine M Manfred [1], Robert Peverall [1], Grant A D Ritchie [1]

Affiliations + expand

PMID: 33769036 DOI: 10.1021/acs.analchem.0c04651

Abstract

Precise optical rotation measurements play an important role in the analysis of chiral molecules in various fields, especially in biological chemistry and pharmacology. In this paper, we demonstrate a new variant of continuous-wave cavity-enhanced polarimetry for detecting the optical activity of two enantiomers of a chiral molecule at 730 nm. It is based on a signal-reversing technique for which the chiral specific rotation is directly determined by the cavity ring-down signal from two counter-propagating beams in a bow-tie cavity. In particular, we ensure reproducible excitation of both modes by broadening the linewidth of a diode laser source by application of a radio frequency perturbation to its injection current. The performance of the polarimeter is demonstrated for the specific rotation of (+)- and (-)-α-pinene in different environments, including the pure vapor, open air, and the liquid phase; the detection precision ranges between 10^{-5} and 10^{-4} degrees per cavity pass depending on the environment. The apparatus is a robust and practical tool for quantifying chirality and can be developed for the entire visible and near-infrared spectral regions.

Anal. Chem. **2004,** *76,* 1184−1188

Polarimetric Assay for the Medium-Throughput Determination of α-Amino Acid Racemase Activity

Dorian L. Schönfeld and Uwe T. Bornscheuer*

Institute of Chemistry & Biochemistry, Department of Technical Chemistry & Biotechnology, Ernst-Moritz-Arndt-University Greifswald, Soldmannstrasse 16, D-17487 Greifswald, Germany

A polarimetric assay has been developed for the identification of α-amino acid racemase activity. The setup consists of a microcuvette polarimeter (40 μL volume) connected to a pipetting robot for microtiter plates, a pump, and data processing. It could be demonstrated for a glutamate racemase from *Lactobacillus fermentii,* expressed in *Escherichia coli*, serving as model enzyme, that its activity can be determined from the time-dependent change of the optical rotation using L-glutamate as substrate. Thus, the specific activity was determined to 111.4 mdeg/min which corresponds to 45.7 μmol/min per mg purified enzyme. Moreover, a protocol was developed that allows the measurement of racemase activity from 96-well microtiter plates using purified enzymes. Thus, the method described can be used to determine racemase activity in an automatic manner. It should be also applicable for the screening of enzyme libraries created by directed evolution.

combinations of hydantoinase, carbamoylase, and a racemase[5,6] and for the synthesis of optically pure mandelic acid.[7]

Modern molecular biology methods, such as cloning and expression of enzymes from "noncultivated" microorganims,[8−10] and the directed evolution[11,12] of proteins boost the number of available biocatalysts or mutants derived therefrom, and consequently, methods for their rapid and reliable characterization are required.

For α-amino acids and α-hydroxy acids, racemase activity can be determined by coupling their activity with an L-specific α-amino acid or alcohol dehydrogenase in connection with a dye.[13] Alternatively, rather expensive techniques based on circular dichroism have been described.[14] To reduce the costs for expensive equipment or the requirement of specific additional enzymes such as dehydrogenases, a generally and broadly applicable method would be desirable. One possibility is the determination of optical rotation values by polarimetric measurements. However, current equipment requires rather large amounts of substrates and enzymes and the measurement of numerous

J. Nepal Chem. Soc., Vol. 36, 2017

Screening of the Physico – Chemical Parameters of Essential Oil *Aegle marmelous* from Dang District of Nepal

Khuma Sharma Dhital*

Department of Chemistry, Patan Multiple Campus, Tribhuvan University, Nepal
Email: khumadhital@gmail.com

Abstract

Aegle marmelos leaves are used as anti-diabetes agent in Ayurveda, Unani & Siddha Systems of Medicine. The essential oil of fresh leaves of Bael (Aegle marmelous) was isolated by hydro-distillation method using Clevenger apparatus and the oil percentage was 1.1 %. Aegle marmelos leaves wild variety from dang district forest were analysed for Specific Gravity, Refractive Index, Optical Rotation, Acid Value and Ester Value. Specific gravity is 0.93 at 20 ˚C; Refractive index is 1.534 at 25 ˚C, Optical rotation -0.09˚ at 23 ˚C, Acid Value 2.4 and Ester Value 17.7.

Key Words: *Bael, Fresh leaves, essential oil, physic-chemical parameters*

ISSN 0030-400X, Optics and Spectroscopy, 2016, Vol. 120, No. 1, pp. 45–49. © Pleiades Publishing, Ltd., 2016.
Original Russian Text © A.B. Pravdin, V.A. Spivak, D.A. Yakovlev, 2016, published in Optika i Spektroskopiya, 2016, Vol. 120, No. 1, pp. 53–58.

On the Possibility of Noninvasive Polarimetric Determination of Glucose Content in Skin

A. B. Pravdin, V. A. Spivak, and D. A. Yakovlev

Saratov State University, Saratov, 410012 Russia

e-mail: pravdinab@mail.ru

Received March 25, 2015

Abstract—Based on real structure and optical properties of the dermis, we analyzed the possibility of polarimetric measurement of glucose content in the skin. It was shown that, at physiological concentrations of glucose in the interstitial fluid, the optical activity of glucose is not manifested in the polarization and optical properties of the tissue, since the optical activity of glucose is almost completely suppressed by the linear birefringence of the dermis.

DOI: 10.1134/S0030400X16010161

Specific Rotation and Carbohydrate Profile of Croatian Unifloral Honeys

Ljiljana PRIMORAC[1], Ivana FLANJAK[1], Daniela KENJERIĆ[1], Dragan BUBALO[2] and Zoran TOPOLNJAK[1]

[1]Department of Food and Nutrition Research, Faculty of Food Technology, University of Osijek, Osijek, Croatia; [2]Faculty of Agriculture, University of Zagreb, Zagreb, Croatia

Abstract

Primorac Lj., Flanjak I., Kenjerić D., Bubalo D., Topolnjak Z. (2011): **Specific rotation and carbohydrate profile of Croatian unifloral honeys**. Czech J. Food Sci., **29**: 515–519.

Specific rotation and carbohydrate profile of Croatian black locust (*Robinia pseudoacacia* L.), sage (*Salvia officinalis* L.) and chestnut (*Castanea sativa* Mill.) honeys were determined. Fructose, glucose, sucrose, maltose (with cellobiose and trehalose), melezitose (with erlose), raffinose, and xylose were evaluated and quantified by HPLC, while specific rotation was determined by using a polarimeter. The differences in the carbohydrate profile, especially in disaccharide and trisaccharide contents, reflected different specific rotation values of the honey types selected. Weak positive correlations between specific rotation and sucrose, melezitose with erlose, and raffinose contents were found.

Keywords: specific rotation; carbohydrate profile; unifloral honey

Alat Uji Kualitas Madu Menggunakan Polarimeter Dan Sensor Warna

Bagus Arief Wibowo, Muhammad Rivai, dan Tasripan
Teknik Elektro, Fakultas Teknologi Industri, Institut Teknologi Sepuluh Nopember (ITS)
Jl. Arief Rahman Hakim, Surabaya 60111 Indonesia
e-mail: muhammad_rivai@ee.its.ac.id

***Abstrak*— Madu merupakan cairan kental menyerupai sirup yang memiliki rasa manis. Rasa manis dalam madu terbentuk secara alami oleh lebah dan serangga yang berasal dari nektar bunga. Kandungan madu didominasi oleh gula (79,8%) dan air (17%). Di lapangan banyak terjadi kasus pemalsuan madu yang mengakibatkan hilangnya sifat manis alami dari madu tersebut. Untuk mengetahui pemalsuan tersebut biasanya dilakukan uji laboratorium yang dirasa kurang efisien. Dalam penelitian ini dilakukan pembuatan alat uji kualitas madu dengan menggunakan polarimeter dan sensor warna untuk mengetahui madu alami atau campuran. Madu mengandung gula yang dapat memutar bidang polarisasi sehingga ketika konsentrasi gula semakin tinggi, maka semakin jauh pula simpangan sudutnya. Rotasi optis yang diamati/diukur bergantung kepada jumlah senyawa dalam tabung madu, panjang jalan/larutan yang dilalui cahaya, temperatur pengukuran, dan panjang gelombang cahaya yang digunakan. Sensor warna berfungsi untuk mengambil data RGB dari 2 jenis madu yang diuji pada alat ini yaitu madu karet dan madu kapas. Berdasarkan pengujian didapatkan bahwa untuk madu karet alami menghasilkan sudut rata-rata -22.67º, madu kapas alami -32.47º. Sedangkan untuk madu karet campuran (madu karet 35 ml+larutan glukosa 10% 35 ml) menghasilkan sudut rata-rata -4.55º, dan madu kapas campuran (madu kapas 35 ml+larutan glukosa 10% 35 ml) dengan sudut rata-rata -17.66º. Sedangkan kemungkinan keberhasilan pendeteksian kualitas untuk madu karet alami sebesar 40%, madu kapas alami 80%, madu karet campuran 100%, dan madu kapas campuran 100%**

***Kata Kunci*—Polarimeter, Sensor Warna, Madu, Glukosa,fruktosa**

menjadi kurang efektif apabila menginginkan diperolehnya data secara langsung dan cepat.

Dengan menggunakan alat ukur polarimeter dapat diketahui kadar gula yang terdapat pada madu. Pada saat konsentrasi gula semakin tinggi, maka cahaya yang tertahan di analisator menjadi lebih redup. Sehingga sudut putar jenisnya pun menjadi semakin besar. Ini menandakan larutan gula dapat membelokan arah getar cahaya. Rotasi optis yang diamati/diukur bergantung kepada jumlah senyawa dalam tabung madu, panjang jalan/larutan yang dilalui cahaya, temperatur pengukuran, dan panjang gelombang cahaya yang digunakan. Dengan tambahan sensor warna dapat diketahui jenis madu tersebut [3]. Modul sensor warna yang digunakan adalah DT Sense Color Sensor menggunakan chip TAOS TCS3200. Modul ini telah terintegrasi dengan 2 LED. Sensor Warna TCS3200 mendeteksi dan mengukur intensitas warna tampak pada madu. Chip TCS3200 memiliki beberapa fotodioda, dengan masing-masing filter warna yaitu, merah, hijau, biru, dan *clear*. Filter-filter tersebut didistribusikan pada masing-masing *array*. Modul ini memiliki osilator yang menghasilkan pulsa kotak yang frekuensinya sebanding dengan perubahan warna yang dideteksi [4].

Dua parameter di atas diharapkan dapat menjadi acuan apakah madu tersebut alami atau campuran. Sehingga dalam penelitian ini telah dibuat sebuah alat uji kualitas madu menggunakan polarimeter dan sensor warna yang dapat mengatasi masalah tersebut. Diharapkan konsumen tidak akan ragu lagi tentang kealamian madu yang dikonsumsinya.

Thank You