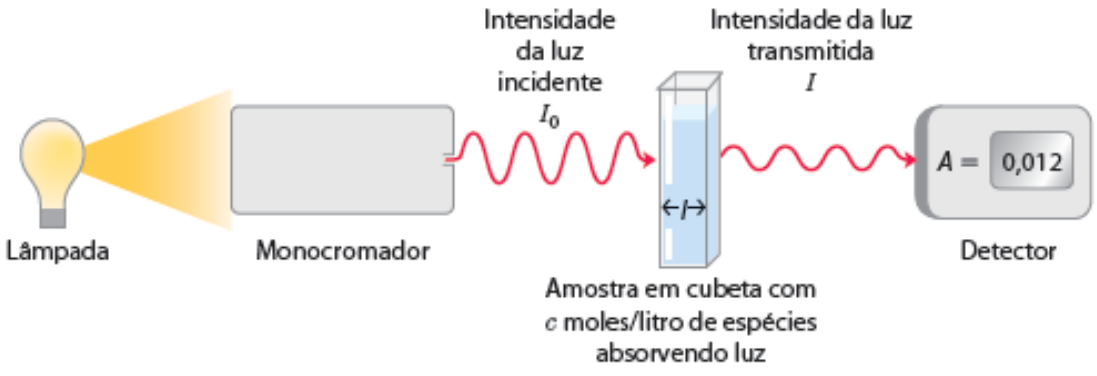
Universidade de São Paulo
Instituto de Química

Prática 1: Espectrofotometria e Colorimetria: Bases teóricas e instruções

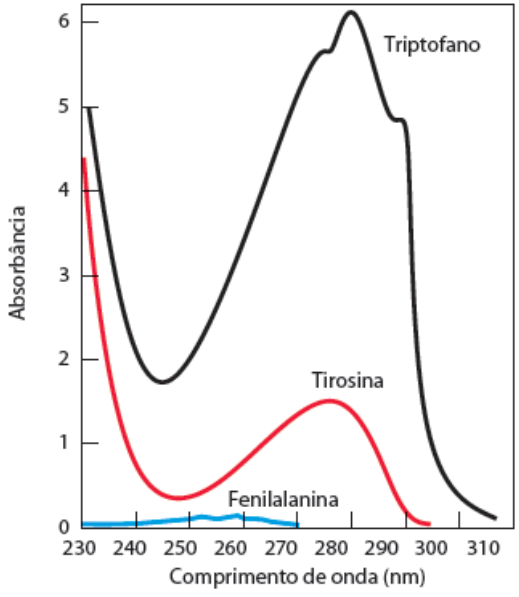
Prof. Henning Ulrich

COLORIMETRIA/ESPECTROSCOPIA

PROCEDIMENTOS ANALÍTICOS



Quantidades Equimolares



LEI DE LAMBERT-BEER

$$\log \frac{I_0}{I} = \varepsilon c l$$

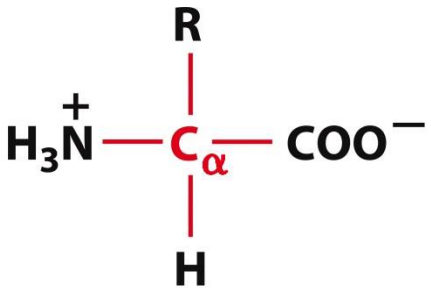
→ Absorbância (A)

I_0 intensidade de luz incidente
I intensidade de luz transmitida
ε coeficiente de extinção molar ou absortividade molar (L/mol/cm)
(característico de cada substância)
c concentração da substância (mol/L)
l comprimento do caminho de luz ou caminho óptico (em cm)

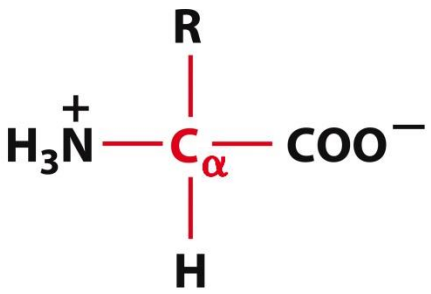
Espectroscopia de Absorção

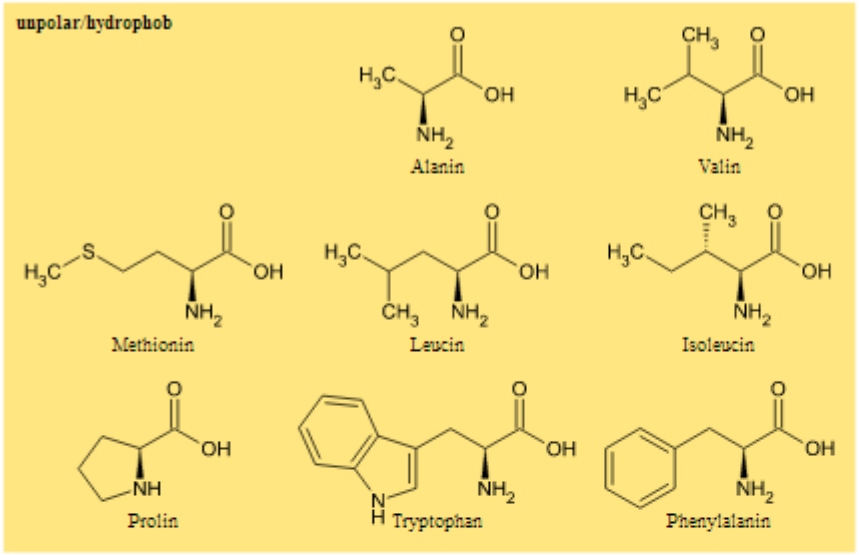
Pela Lei de Beer podemos concluir que a absorbância de uma solução é diretamente proporcional à concentração da espécie absorvente quando se fixa o comprimento

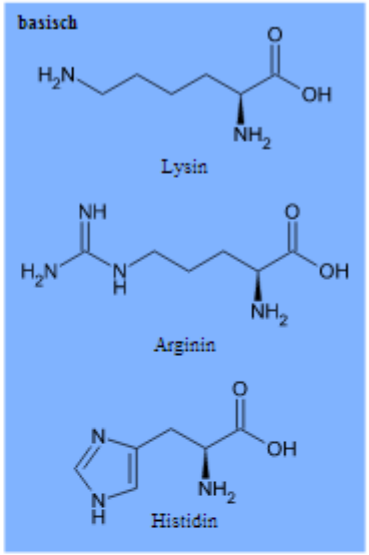
AMINOÁCIDOS

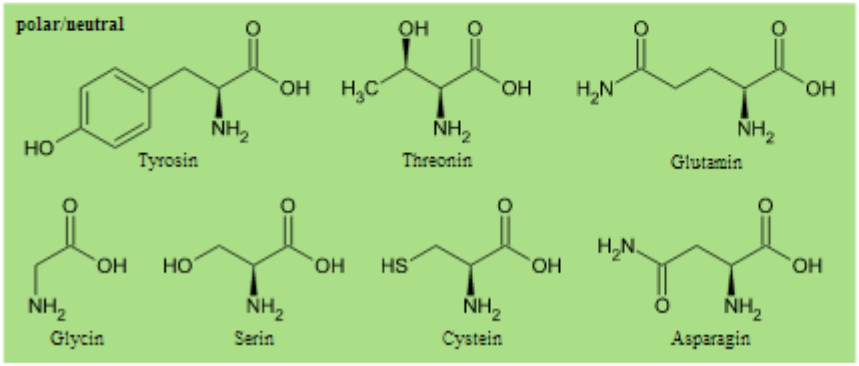


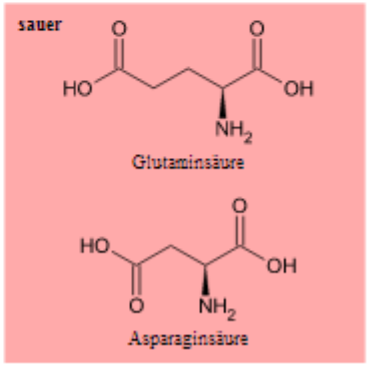
AMINOÁCIDOS



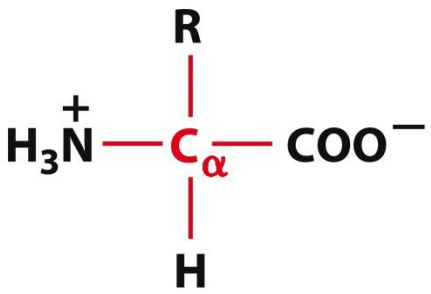


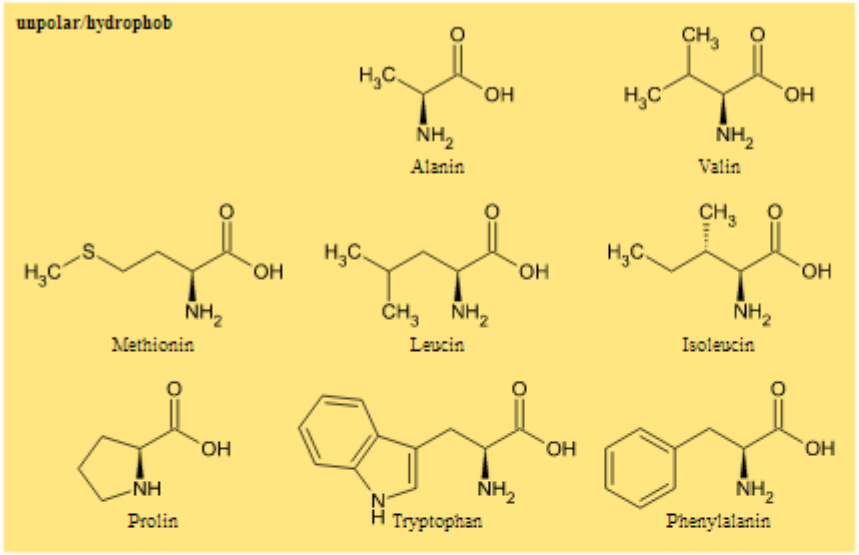


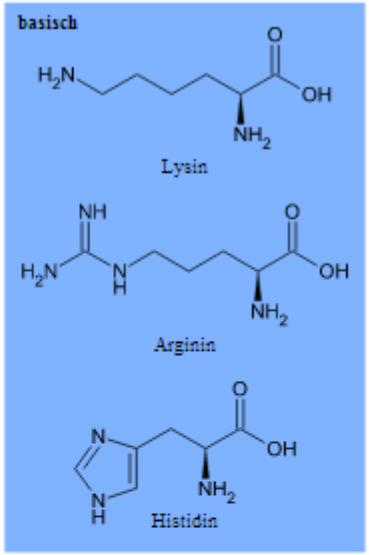


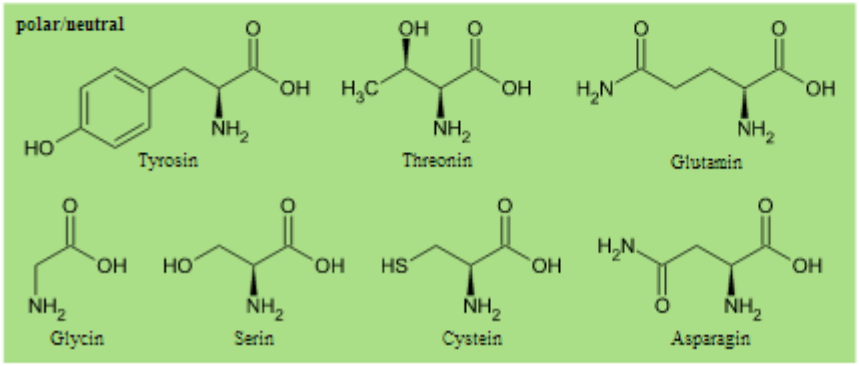


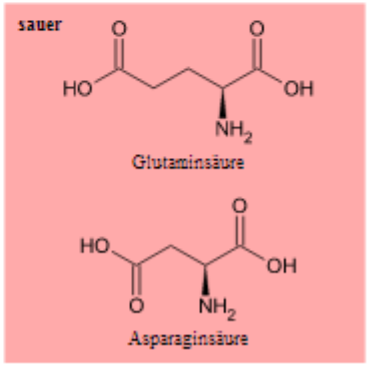
AMINOÁCIDOS



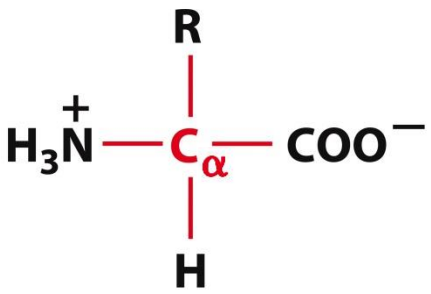


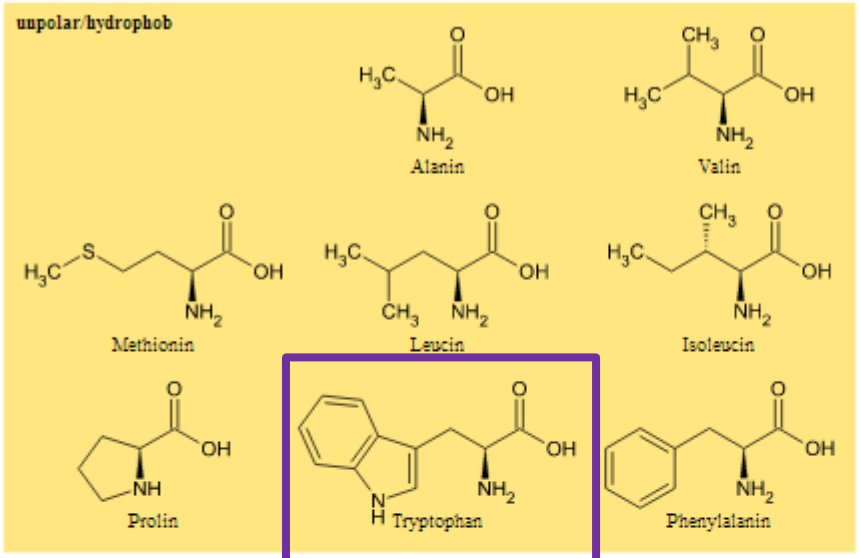


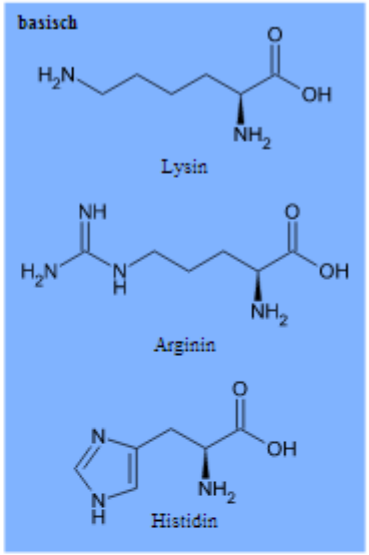


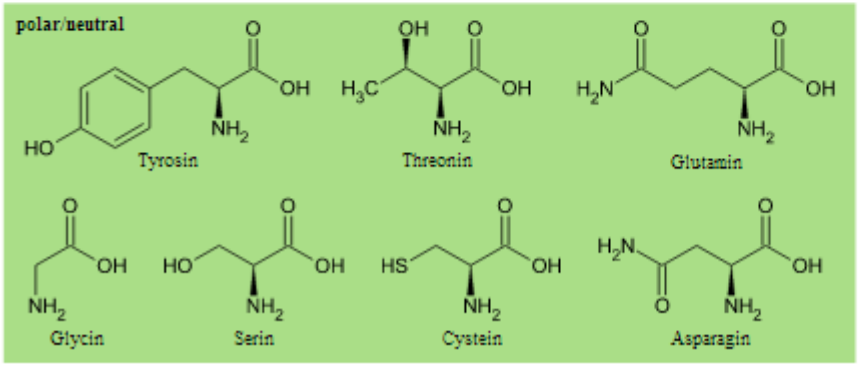


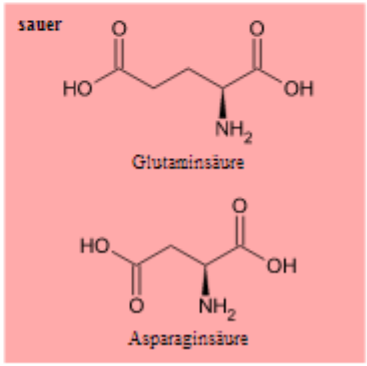
AMINOÁCIDOS



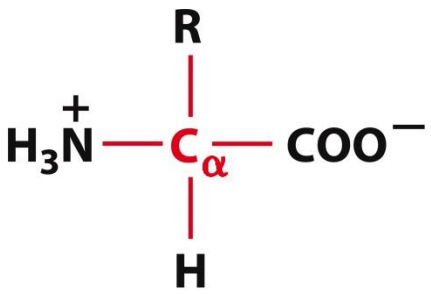


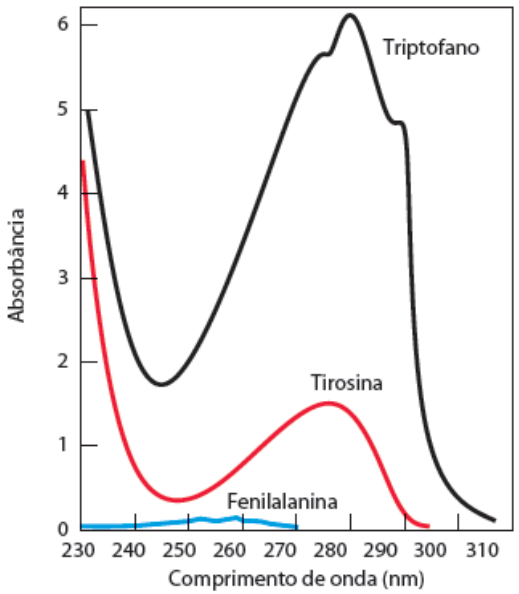


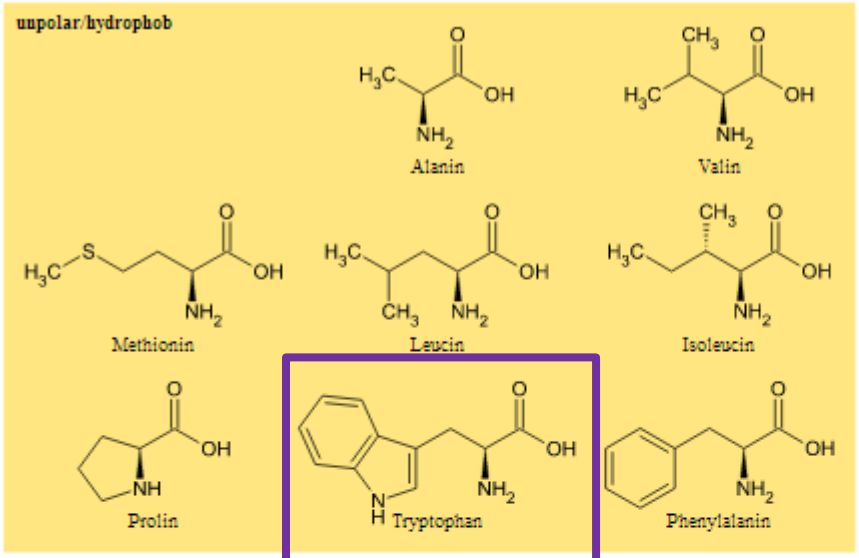


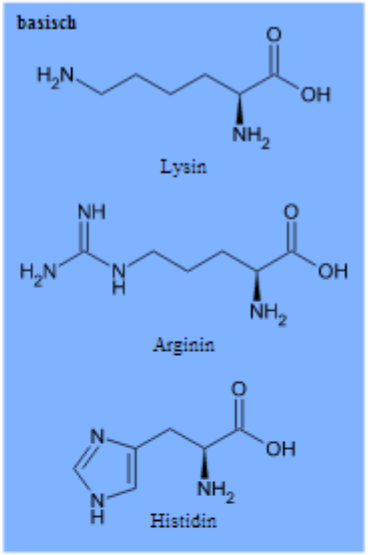


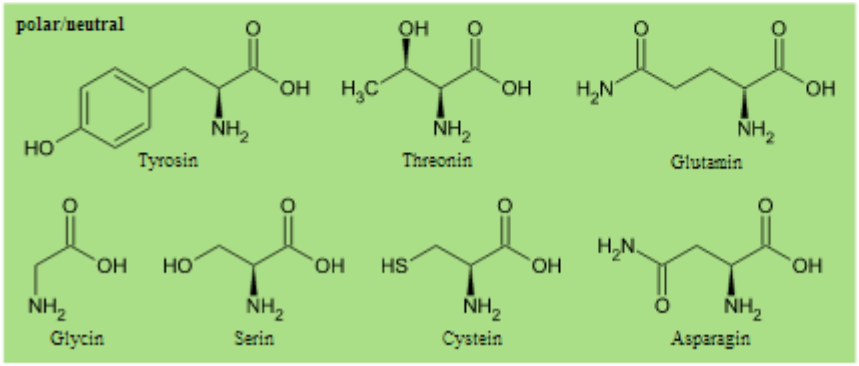
AMINOÁCIDOS











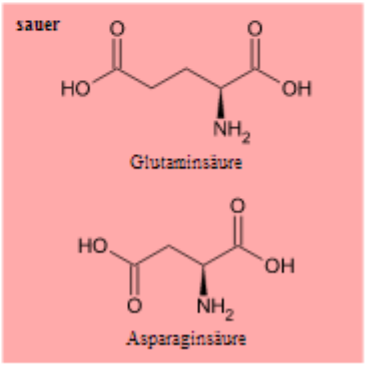
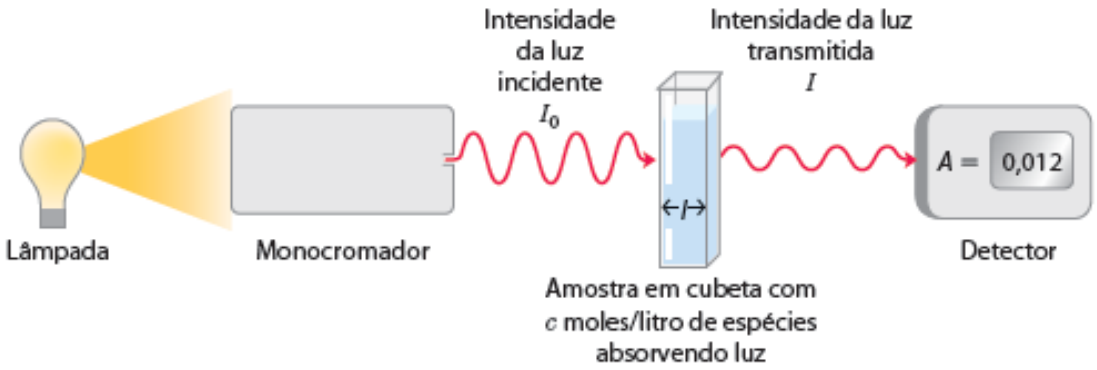
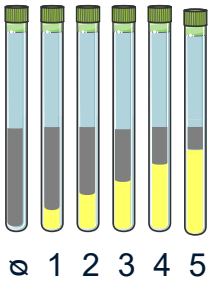


Figura de Princípios de Bioquímica de Lehninger

COLORIMETRIA/ESPECTROSCOPIA

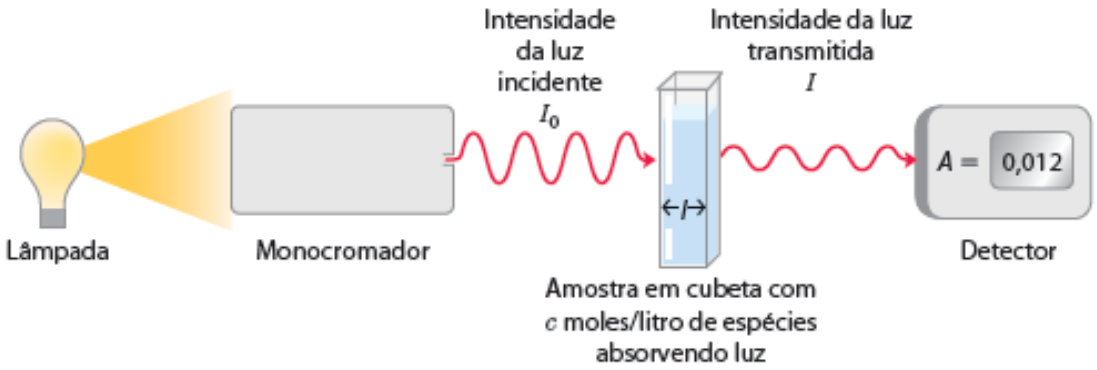
PROCEDIMENTOS ANALÍTICOS

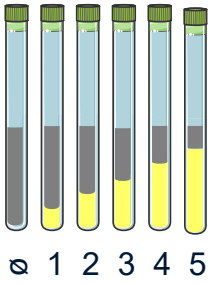


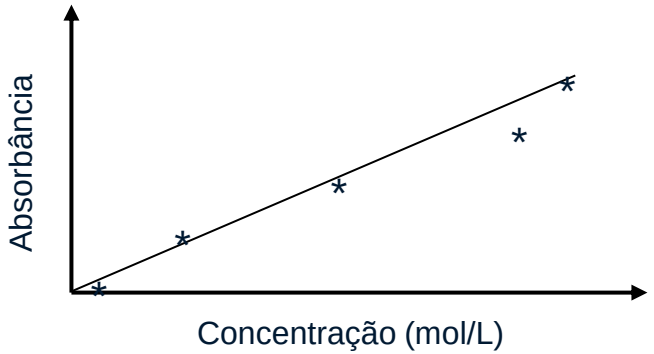


COLORIMETRIA/ESPECTROSCOPIA

PROCEDIMENTOS ANALÍTICOS

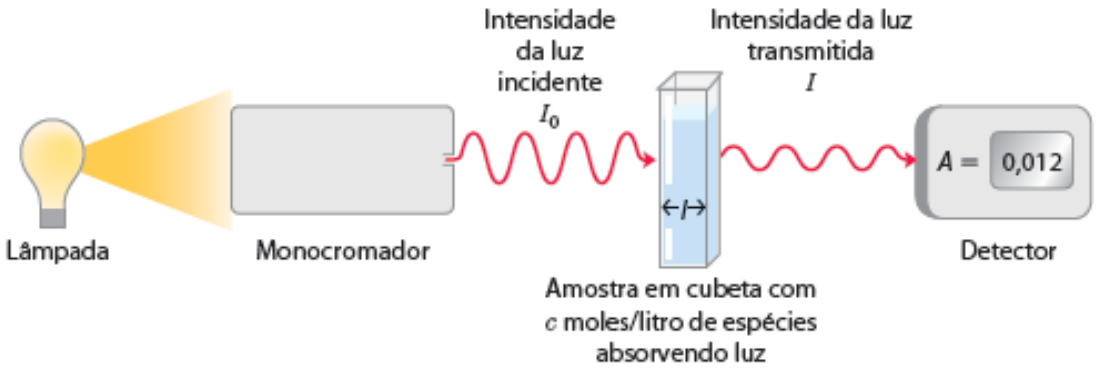


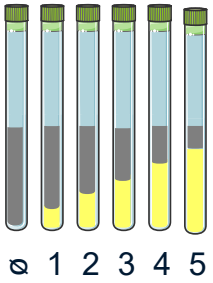




COLORIMETRIA/ESPECTROSCOPIA

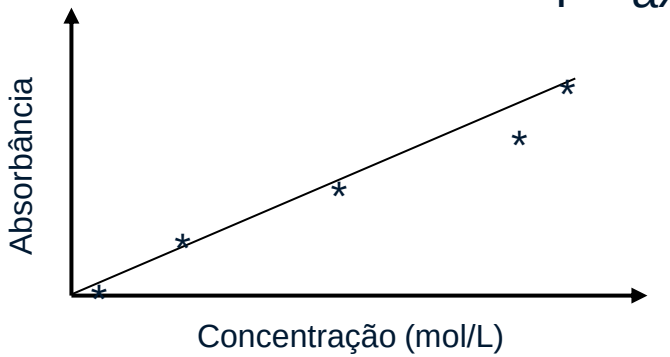
PROCEDIMENTOS ANALÍTICOS





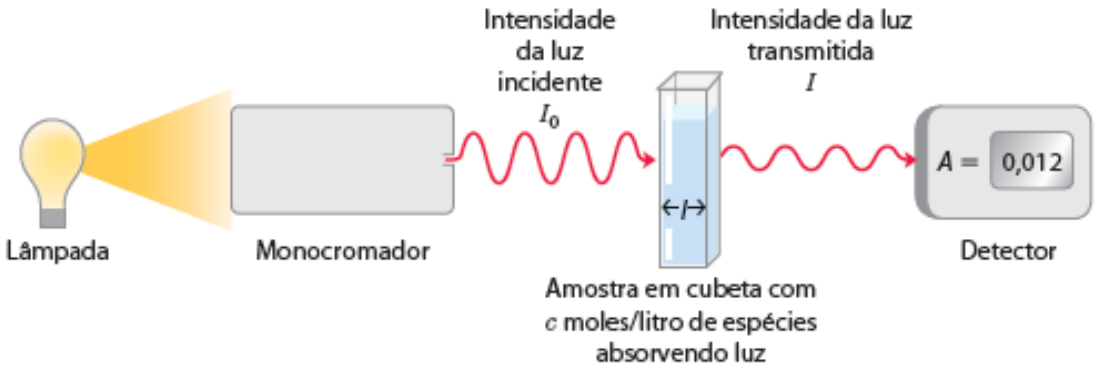
R^2

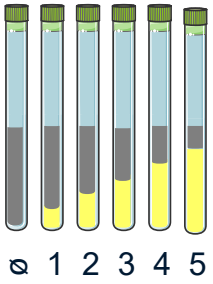
$Y = ax+b$



COLORIMETRIA/ESPECTROSCOPIA

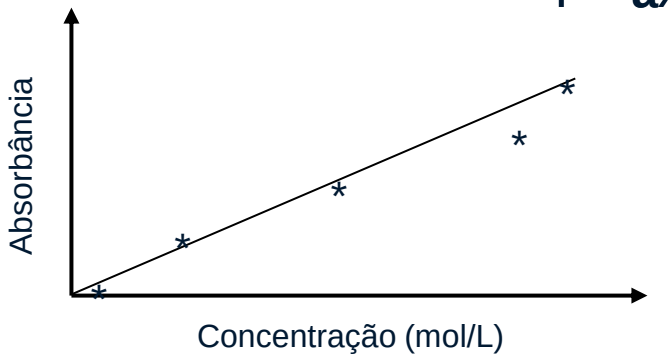
PROCEDIMENTOS ANALÍTICOS





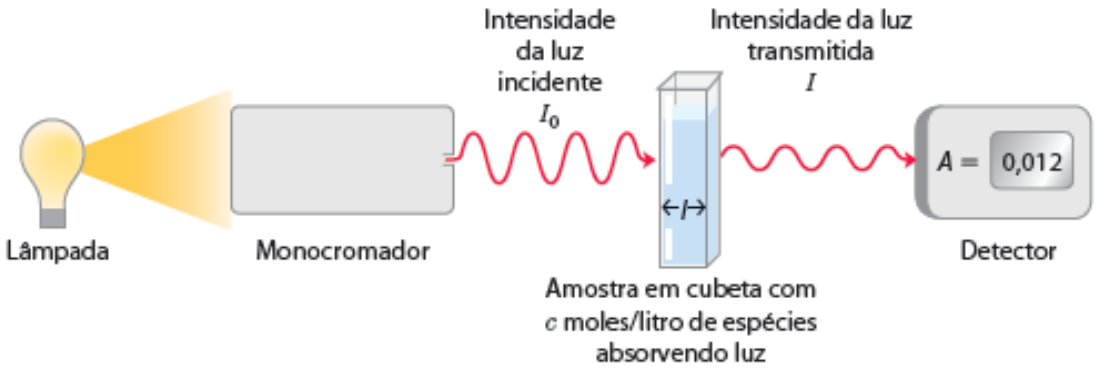
R^2

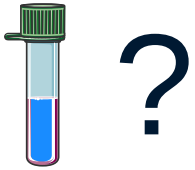
$Y = \mathbf{a}x + \mathbf{b}$



COLORIMETRIA/ESPECTROSCOPIA

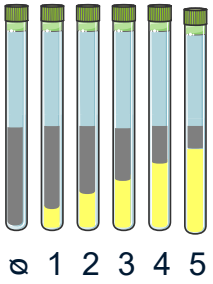
PROCEDIMENTOS ANALÍTICOS

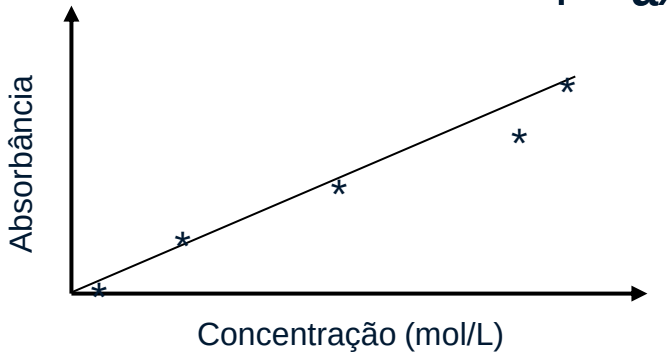




R^2

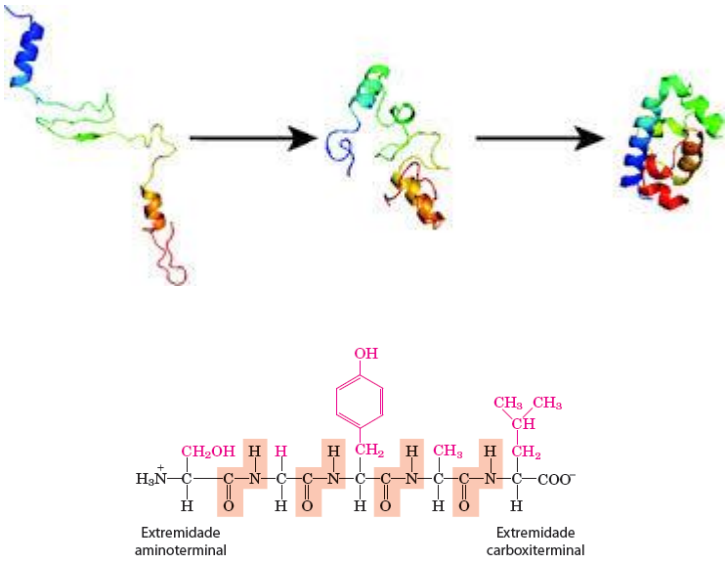
$Y = ax+b$

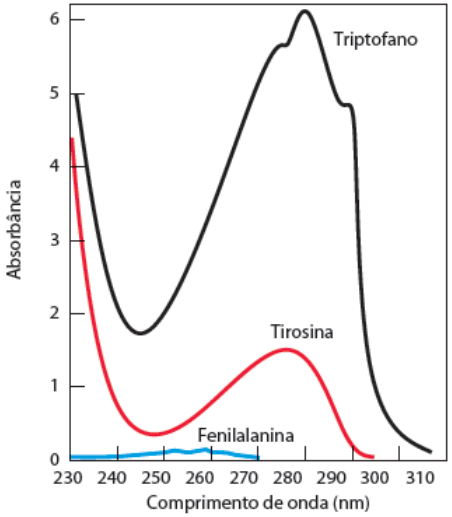


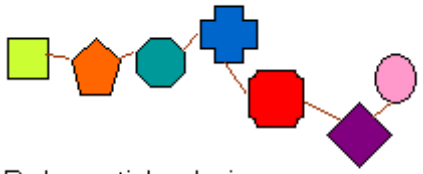


COLORIMETRIA/ESPECTROSCOPIA

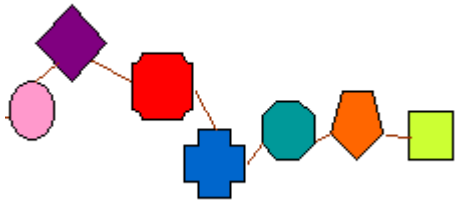
PROCEDIMENTOS ANALÍTICOS

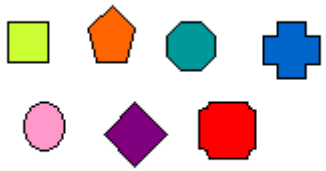






Polypeptide chain - a short chain of amino acids

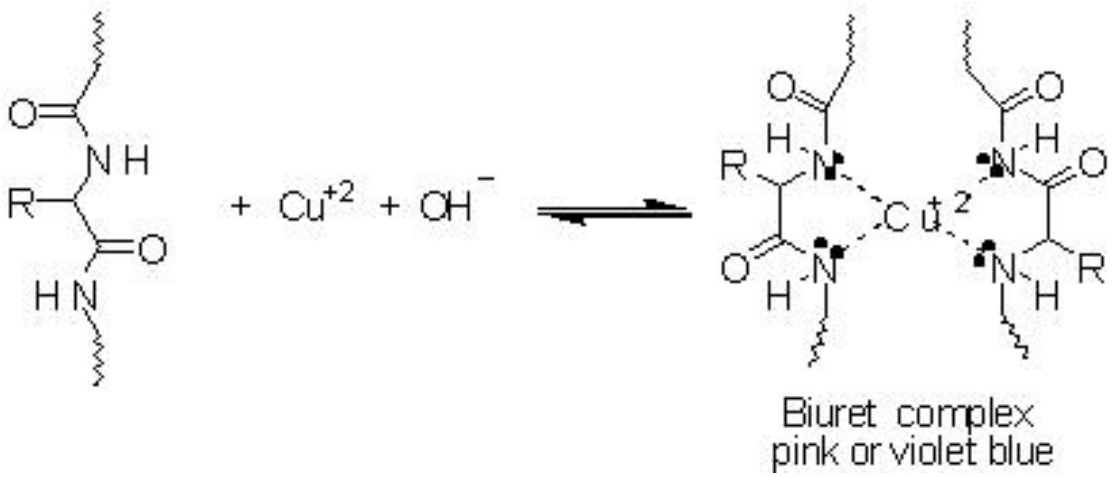


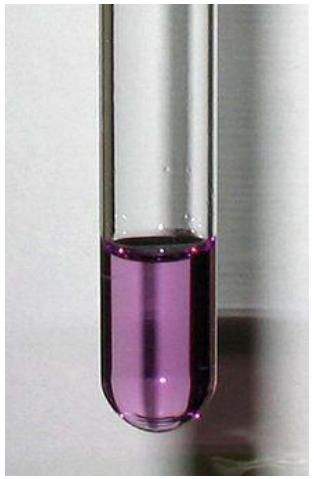


Amino acids

COLORIMETRIA/ESPECTROSCOPIA

REAÇÃO DE BIURETO





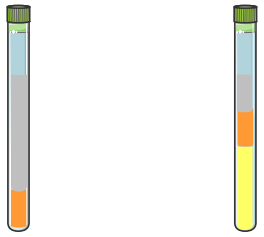
Reação de biureto
(Wikipedia)

AULA PRÁTICA - 1

OBJETIVOS

1) DETERMINAÇÃO DA CONCENTRAÇÃO DE UMA PROTEÍNA

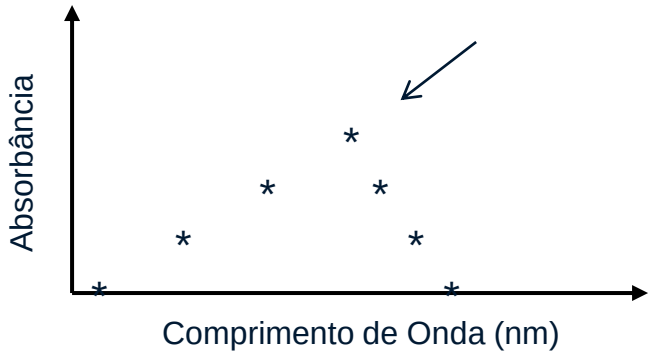
A) DETERMINAÇÃO DO λMÁX DO PRODUTO DA REAÇÃO DE BIURETO;

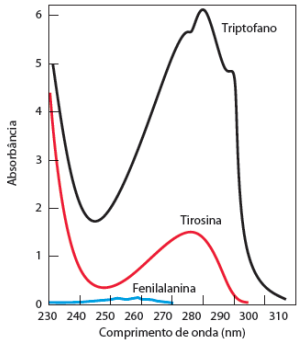


Branco
(só biureto)

Albumina
+ biureto

15 min a 37ºC





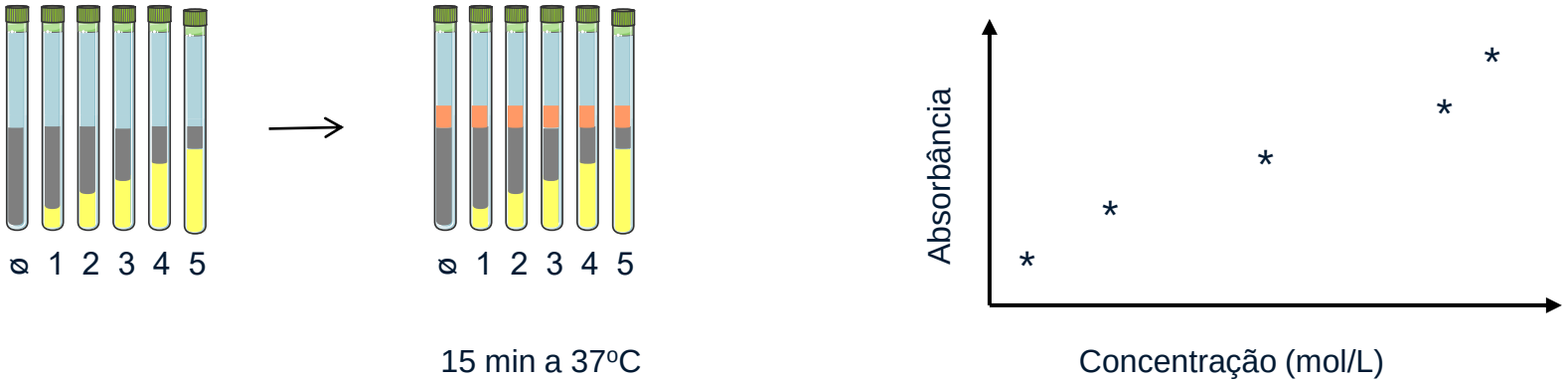
400, 420, 450, 470, 500, 520, 550, 580, 600, 630, 650, 680 e 700 nm

AULA PRÁTICA - 1

OBJETIVOS

1) DETERMINAÇÃO DA CONCENTRAÇÃO DE UMA PROTEÍNA

B) DETERMINAÇÃO DA CONCENTRAÇÃO DE PROTEÍNA



540 nm

AULA PRÁTICA - 1

OBJETIVOS

1) DETERMINAÇÃO DA CONCENTRAÇÃO DE UMA PROTEÍNA

B) DETERMINAÇÃO DA CONCENTRAÇÃO DE PROTEÍNA

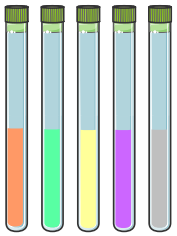
TUBOS	PADRÃO ALBUMINA (8MG/ML)	PROTEÍNA X	ÁGUA DESTILADA	REAGENTE BIURETO	CONCENTRAÇÃO (MG/ML)	ABSORBÂNCIA (540 NM)
BRANCO	-	-	1,5 ML	2,5 ML		
1	0,1ML	-	1,4 ML	2,5 ML		
2	0,2ML	-	1,3 ML	2,5 ML		
3	0,4ML	-	1,1 ML	2,5 ML		
4	0,7ML	-	0,8 ML	2,5 ML		
5	1,0ML	-	0,5 ML	2,5 ML		
X	-	1,0 ML	0,5 ML	2,5 ML		

AULA PRÁTICA - 1

OBJETIVOS

2) DETERMINAÇÃO DO ESPECTRO DE ABSORÇÃO DE LUZ DE AMINOÁCIDOS E BASES PURÍNICAS E PIRIMIDÍNICAS

A) DETERMINAÇÃO DA λMÁX



LEUCINA (0,2 MG/ML)
TRIPTOFANO (0,004 MG/ML)
TIROSINA (0,1 MG/ML)
ADENINA (0,004 MG/ML)
TIMINA (0,01 MG/ML)

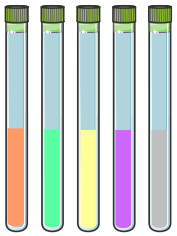
A') $\lambda_{MÁX}$,
A") ABSORBÂNCIA OBTIDA EM $\lambda_{MÁX}$,
A"') CALCULAR ε DE CADA SUBSTÂNCIA

AULA PRÁTICA - 1

OBJETIVOS

2) DETERMINAÇÃO DO ESPECTRO DE ABSORÇÃO DE LUZ DE AMINOÁCIDOS E BASES PURÍNICAS E PIRIMIDÍNICAS

A) DETERMINAÇÃO DA λMÁX



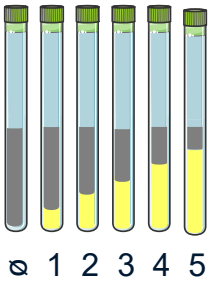
LEUCINA (0,2 MG/ML)
TRIPTOFANO (0,004 MG/ML)
TIROSINA (0,1 MG/ML)
ADENINA (0,004 MG/ML)
TIMINA (0,01 MG/ML)

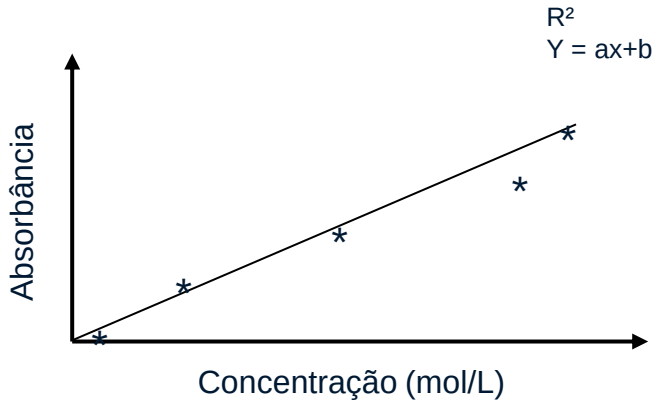
A') $\lambda_{MÁX}$,
A") ABSORBÂNCIA OBTIDA EM $\lambda_{MÁX}$,
A"') CALCULAR ε DE CADA SUBSTÂNCIA

AULA PRÁTICA - 1

OBJETIVOS

2) DETERMINAÇÃO DA CURVA DE ABSORBÂNCIA X CONCENTRAÇÃO





? nm

TUBOS	TRIPTOFANO (0,01 MG/ML)	ÁGUA DESTILADA	CONCENTRAÇÃO (MG/ML)	ABSORBÂNCIA OBTIDA
BRANCO	-	2,0 ML		
1	0,1 ML	1,9 ML		
2	0,2 ML	1,8 ML		
3	0,4 ML	1,6 ML		
4	0,7 ML	1,3 ML		

CORREÇÃO RELATÓRIOS

NOTAS

INTRODUÇÃO …….. 0,5
OBJETIVOS ……..... 0,5
MATERIAL E MÉTODOS…... 0,5
RESULTADOS …… 2,0
CONCLUSÃO ……..……...1,5

QUESTÕES …….5,0