Biochemistry II - Seminars

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Literature

- **Printed handouts with questions** – will be given **before seminar**
- **Seminar ppt files with answers** – uploaded into IS **after seminar**

Spring 2008 → VSBC041s Biochemistry II-s → Study materials → Work with study materials

- **Laboratory manual: Biochemistry II – Practicals 2008**
- **Lectures** – ppt files available at is.muni.cz (VSBC04p)
- **Textbooks:** R. K. Murray et al.: Harper´s Illustrated Biochemistry.

  P. C. Champe, R. A. Harvey: Biochemistry.

Conditions for the credit:

see the back side of the syllabus !!

- 100% attendance
- If you are absent – written elaboration of the chapter must be given to teacher ASAP
- at least 70 % from three revision tests

Optical and electrophoretic methods in clinical chemistry

Seminar No. 1

Spectrophotometry

Q. 1

A. 1

- 180 – 400 nm UV
- 400 – 800 nm VIS

Q. 2

A. 2

- $A = \varepsilon\, c\, l$

- Conditions:
- Monochromatic light
- Diluted solution (< 0.01 mol/l)
- Homogeneous solution
- Monomeric substances which do not exhibit fluorescence

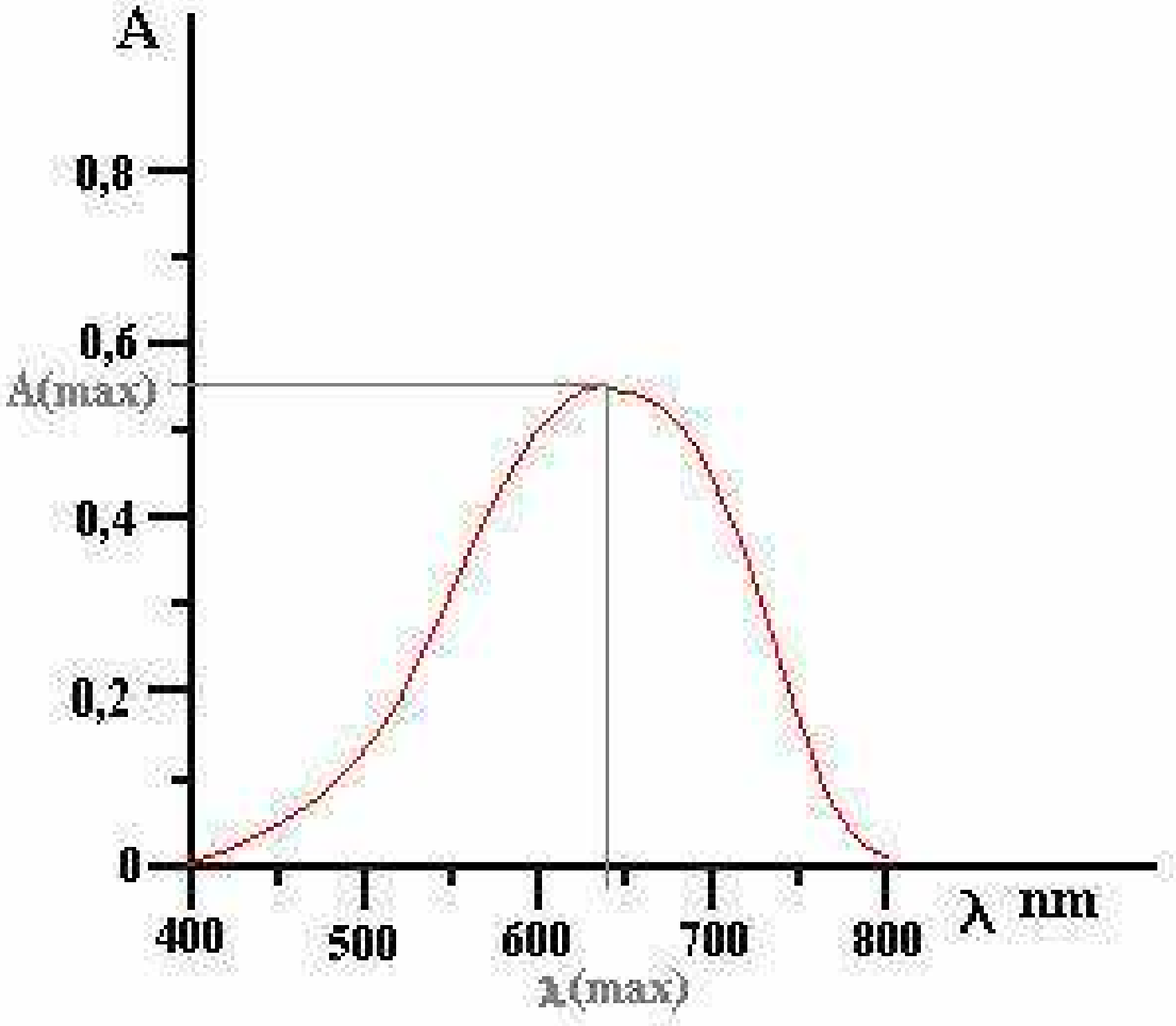
Q. 3

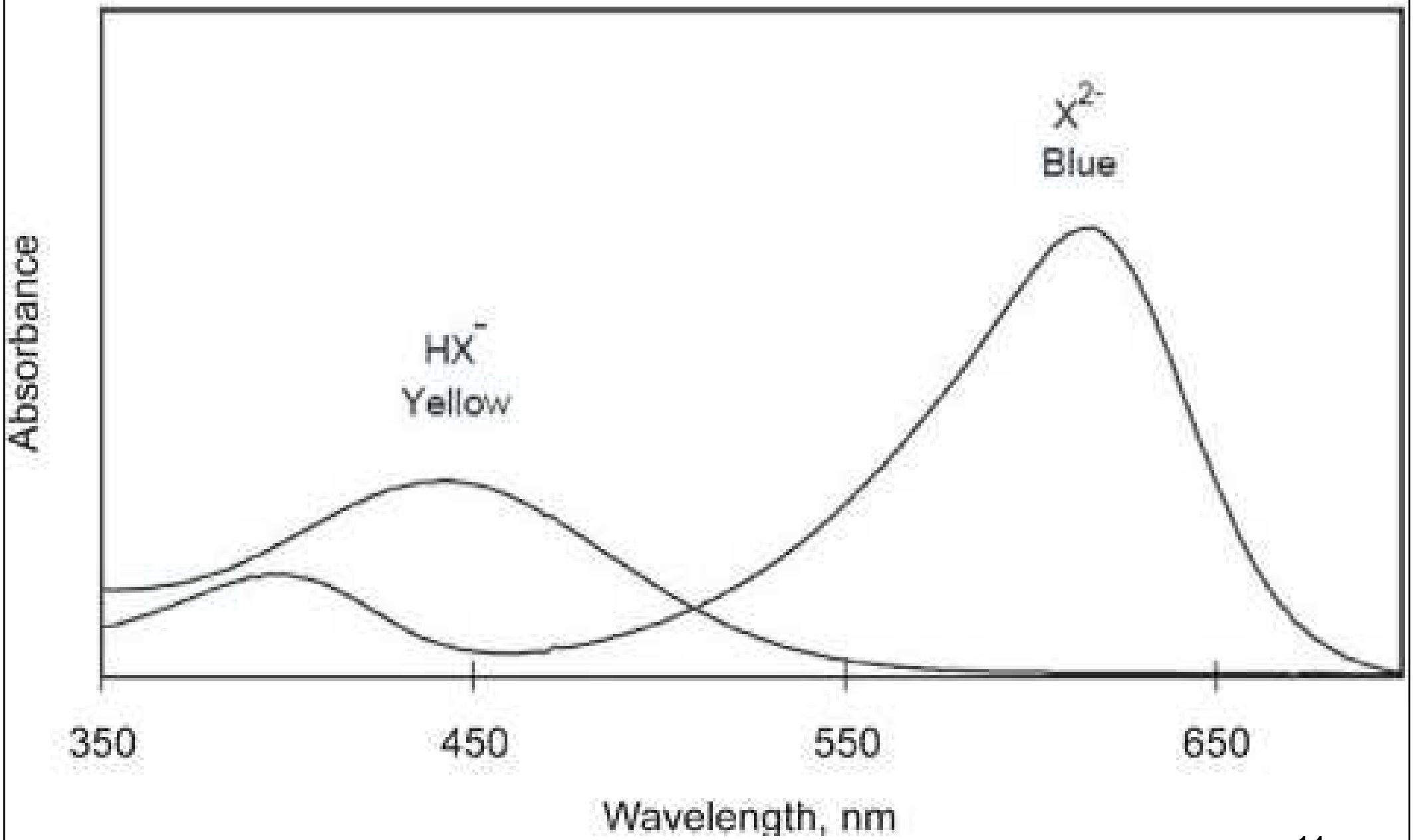
A. 3

- Molar absorption coefficient

- l/mol. cm

Q. 4


A
0,8
0,6
A(max)
0,4
0,2
0
400
500
600
700
800
λ nm
λ(max)

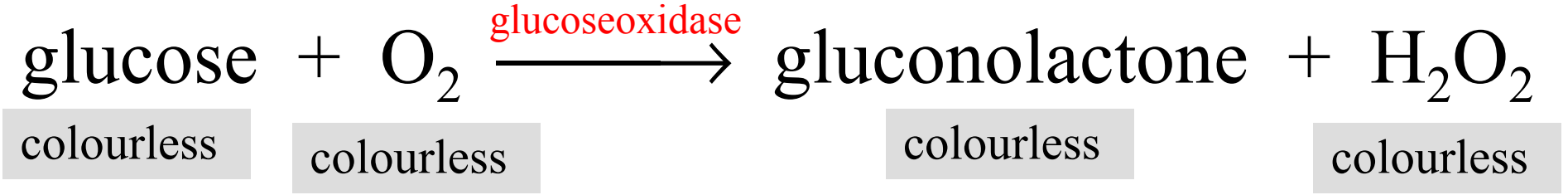

X^{2-}
Blue
HX^-
Yellow
Absorbance
350
450
550
650
Wavelength, nm

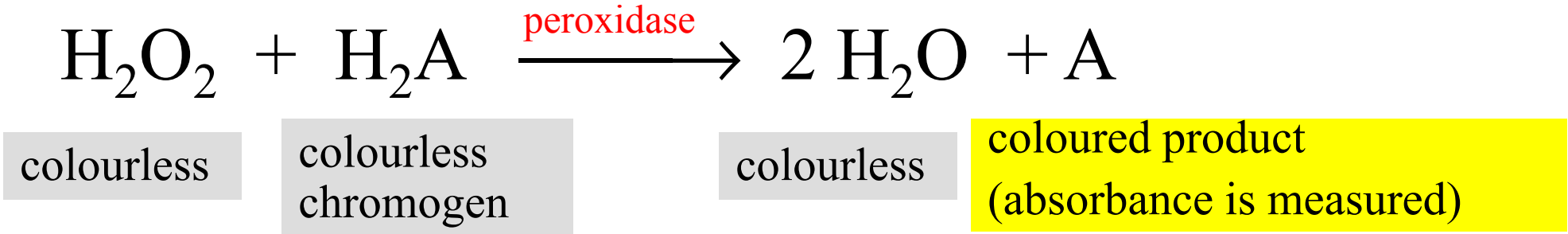
Q. 5

A. 5

- Only coloured compounds

- Colourless compounds (e.g. glucose, cholesterol) have to be converted to coloured derivative by chemical reaction


glucose + O_2
glucoseoxidase
gluconolactone + H_2O_2
colourless
colourless
colourless
colourless


H_2O_2 + H_2A
peroxidase
2 H_2O + A
colourless
colourless chromogen
colourless
coloured product
(absorbance is measured)

Q. 6

A. 6

- Solution which contains everything except the compound to be analyzed

- Solvent (water) + reagent + (buffer) .....

Q. 7

A. 7

a) $c = A / \varepsilon l$

b) $c = A \times$ calibration factor

obtaned as the slope of calibration curve

c) $c = A\, c_{ST} / A_{ST}$

Q. 8

A. 8

$$A = \log 1/T = -\log T$$

$T = 10\ \% = 10 \times 1/100 = 0.1 \Rightarrow$ **A = -log 0.1 = 1**

$T = 50\ \% = 0.5 \Rightarrow$ **A = 0.3**

$T = 100\ \% = 1 \Rightarrow$ **A = 0**

Q. 9

A. 9

$$c = A / \varepsilon = 0.54 / 13900 = 0.0000388 \text{ mol/l}$$

$$= 0.039 \text{ mmol/l}$$

$$= \mathbf{39\ \mu mol/l}$$

Q. 10

A. 10

$\varepsilon = A / c = 0.805 / 0.0005 = 1610$ l/mol.cm

Q. 11

A. 11

Absorbance is additive quantity

Absorbances of sample and standard have to be corrected by the absorbance of reagent

A_{STD} = 0.39 - 0.1 = 0.29 ..... 5 mmol/l

A_{sample} = 0.54 - 0.1 = 0.44 ..... x mmol/l

x = (0.44 × 5) / 0.29 = 7.58 mmol/l

Q. 12

A. 12

0,50 ..... 5 mmol/l

0,75 x

$X = (0{,}75 \times 5) / 0{,}50 = 7.5$ mmol/l

Q. 13

A. 13

Common feature:

Light interacts with a colloidal solution of proteins

Light is scattered on particles, absorption is minimal

Intensity of scattered ligth (*I*) is measured

Difference:

Turbidimetry – *I* measured in the same direction

Nephelometry – *I* measured in perperdicular direction (90°)

Q. 14

A. 14

- The best scatter of light is when the wavelength is close to the size of dispersed particles

- Consider red ligth (500 nm) in fog

Electrophoresis

Q. 15

A. 15

- The pH of solution (buffer)
- The nature of protein – the ratio of acidic an basic AA

Q. 16

Factor / its change	Mobility change
Potential ↑	
Molecular size ↑	
Charge ↑	
pH	
Medium	
Temperature ↑	

A. 16

Factor / its change	Mobility change
Potential ↑	↑
Molecular size ↑	↓
Charge ↑	↑
pH	different
medium	different
Temperature ↑	↑

Q. 17

A. 17

- Elevated alfa-2 and beta globulins
- Contain proteins of acute phase – indicators of acute inflammation

Q. 19

Protein	Function / Feature
Transthyretin	Transport of T4
Albumin	Transport, buffer, oncotic pressure
Alfa1-glycoprot.	Acute phase protein
Alfa1-antitrypsin	Anti-protease
HDL	Transport of cholesterol to liver
Cerulolasmin	Transport of copper
Haptoglobin	Transport of free hemoglobin
Ferritin	Liver prot., in plasma acute phase protein
Alfa2-macroglob.	Acute phase protein
Hemopexin	Transport of free heme
Transferrin	Transport of Fe^{3+}, acute phase protein
CRP	Acute phase protein
Fibrinogen	Blood clotting, acute phase protein
LDL	Transport of cholestrol to tissues
Ig	antibodies

Q. 20

A. 20

- Casein is the main milk protein
- Phosphoprotein – rather acidic – pI = 4.5
- At pH 8.6 it becomes polyanion, goes to + electrode

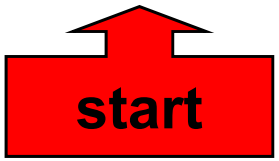
Q. 21

pH = 9.7, when pH = pK_A $\Rightarrow$ 50 % dissociation !!!

AA	Charges of α-COOH	α-NH_3^+	side chain	Total charge
Glutamate				
Isoleucine				
Lysine				

AA	Charges of α-COOH	α-NH_3^+	side chain	Total charge
Glu	**1 -**	**0.5 +**	**1 -**	**1.5 -**
Ile	**1 -**	**0.5 +**	**none**	**0.5 -**
Lys	**1 -**	**0**	**1 +**	**0**

+ ...Glu .....Ile..... Lys ..... -



Q. 23

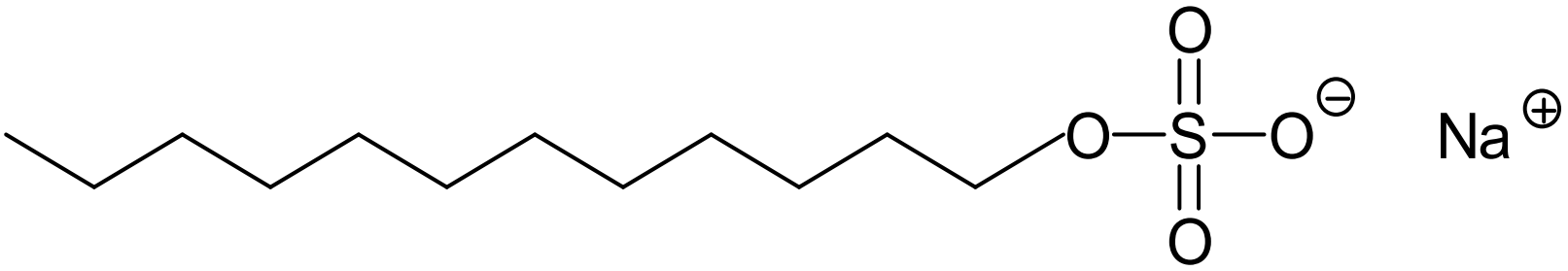
A. 23

a thiol is a reducing agent

reduces disulfide bonds to separate polypetide chains

Q. 24

A. 24



SDS

sodium dodecyl sulfate (SDS) is an anionic surfactant

Q. 26

A. 26

Two separations are performed in two dimensions

The second separation is carried out at 90° to the direction of the first run