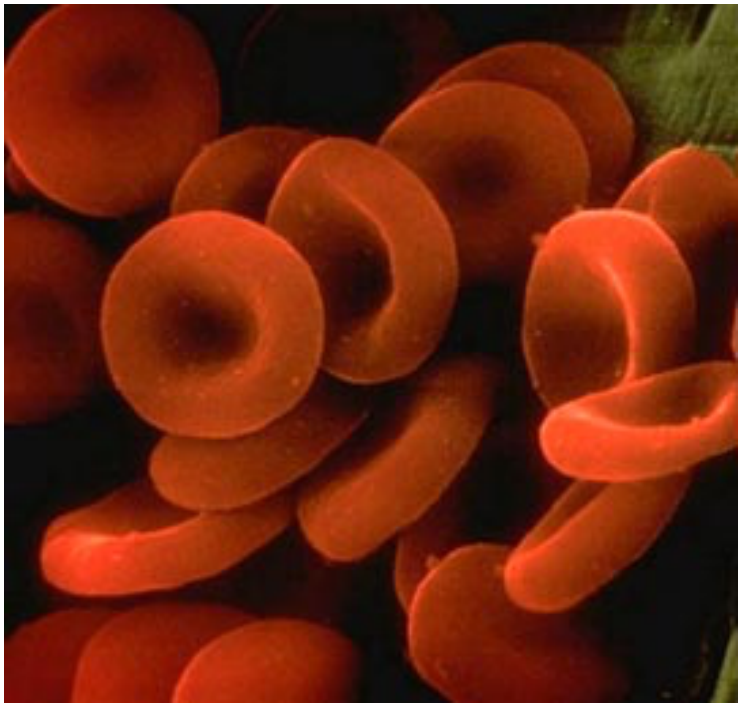
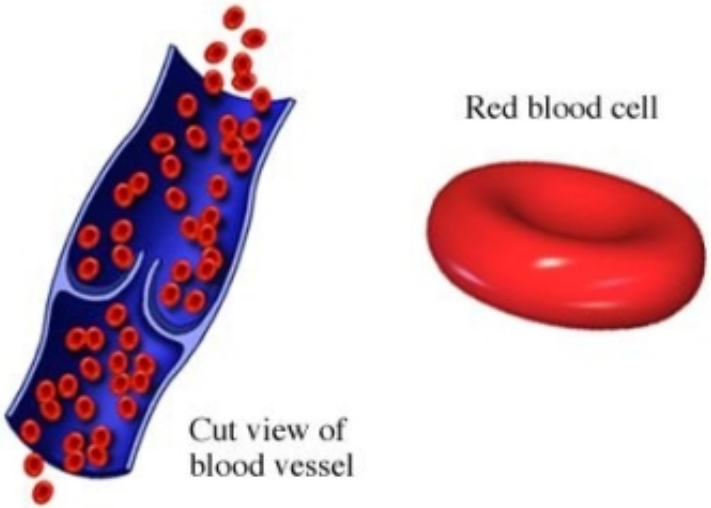
Osmometry and hemolysis

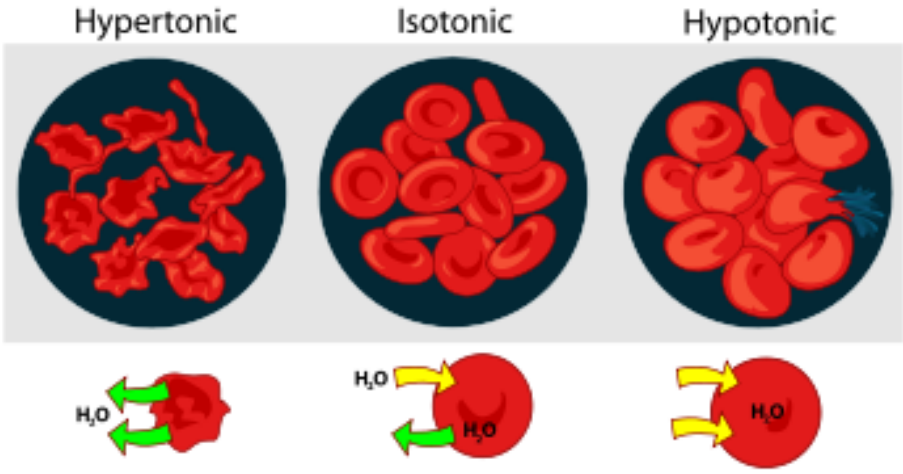


MEASUREMENT OF THE RESISTANCE TO OSMOTIC SHOCK OF RED BLOOD CELLS.

The red blood cell is a small blood cell responsible for the transport of oxygen and devoid of nucleus and organelles. To perform its function, the red blood cell (RBC) contains a high concentration of hemoglobin, a protein whose function is to combine reversibly with oxygen. The red blood cell can not reproduce itself and its average life is 120 days, new red blood cells are constantly produced in the bone marrow stem cells from specialized cellular (erythroblasts).

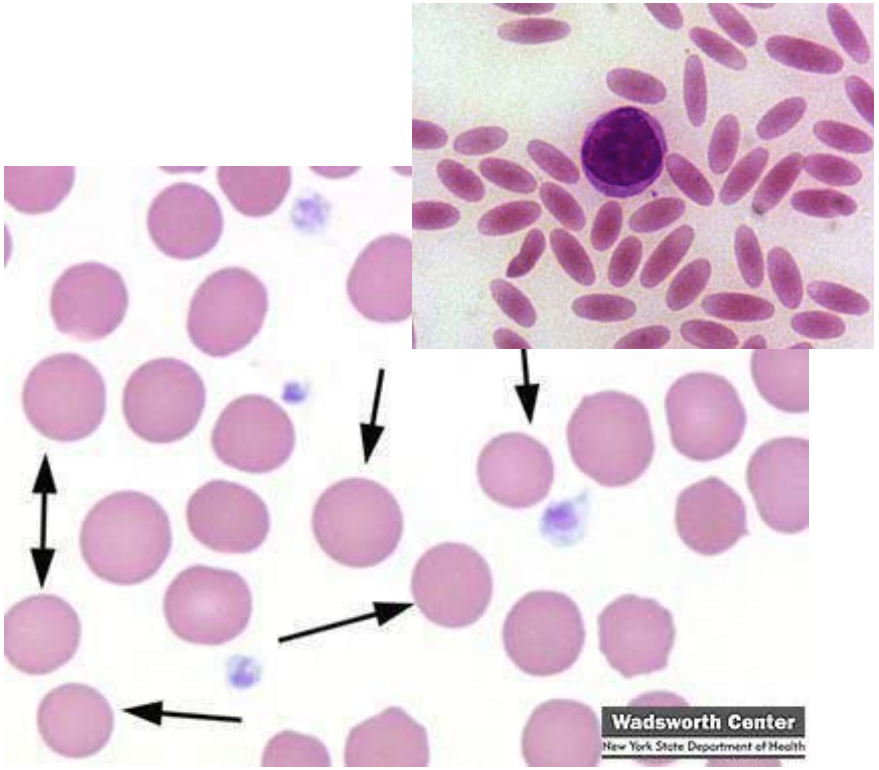


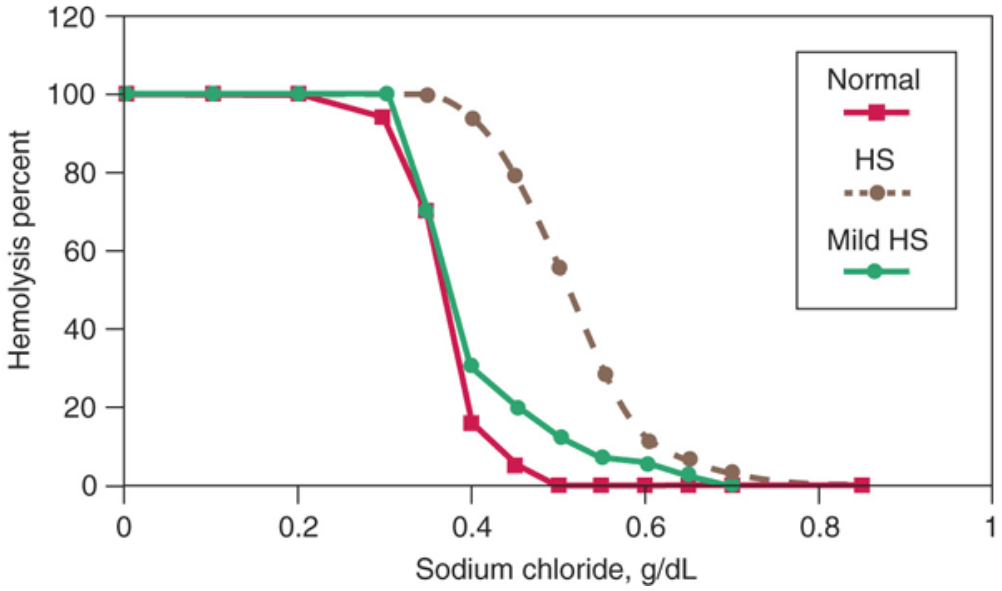
The concentration of solutes in the cytoplasm of the red blood cell is the same as in blood plasma and it exercises an osmotic pressure of about 7.6 atm at 37 ° C. This is the osmotic pressure exerted by 0.15 M NaCl and corresponds to 300 mmol of solute osmotically active per liter (300 mOsm).
Since cell membranes are semipermeable and can be crossed by water, if RBCs are suspended in solutions at lower osmolarity, they absorb water and swell until they burst.

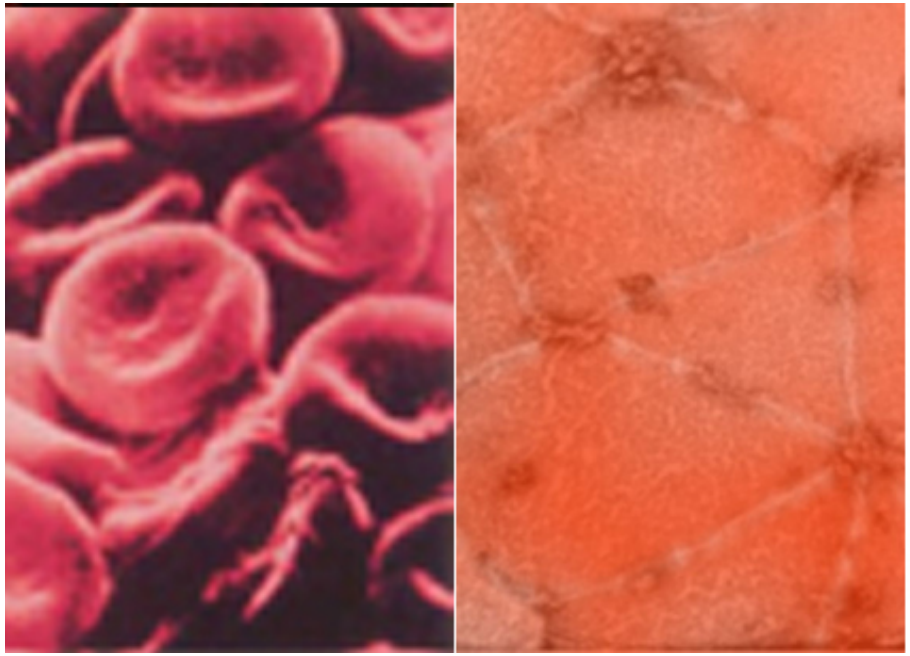


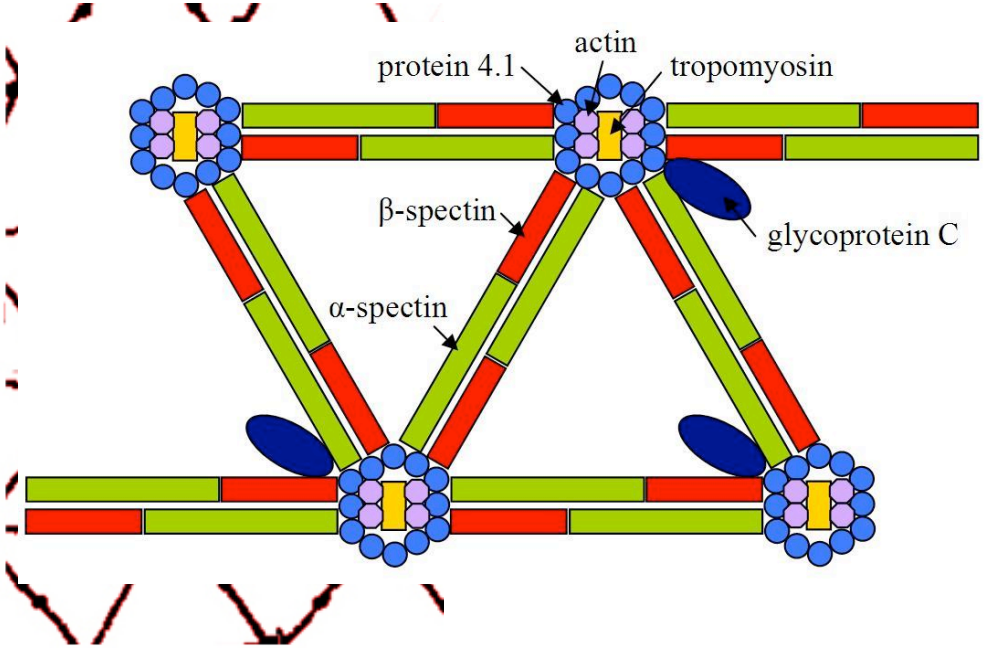
It is possible to measure the resistance of the membrane of red blood cells by suspending them in solutions gradually of decreasing concentration and measuring the fraction of cells that undergo osmotic lysis.
The easiest way to quantify the results of an experiment of this kind is to centrifuge the cells and determine the concentration of hemoglobin in the supernatant.
In fact, the osmotic lysis releases the hemoglobin in red blood cells and the concentration of this protein in the supernatant is directly proportional to the number of cells that have undergone lysis.

There are diseases in which, for defects in the membrane or in the enzyme content in the cytoplasm, the resistance of red blood cells to osmotic stress is decreased. The resulting clinical picture is that of a hemolytic anemia (spurious clinical picture, which may be due to many different causes, not all related to the osmotic resistance of the membrane). Examples of inherited defects in the membrane of red blood are cell spherocytosis and elliptocytosis.

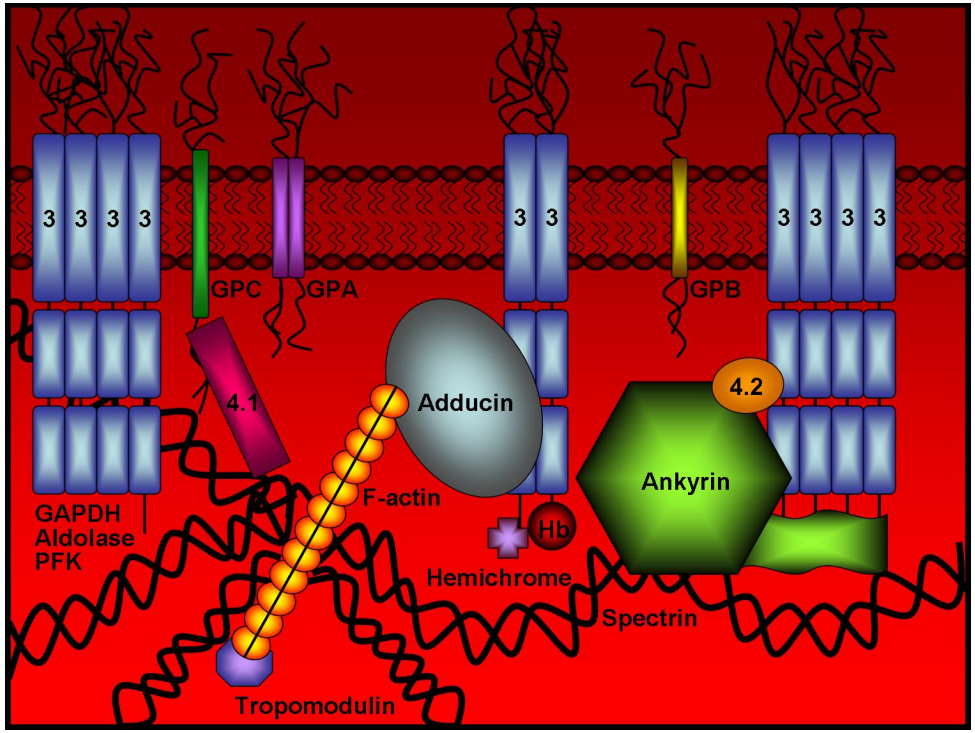


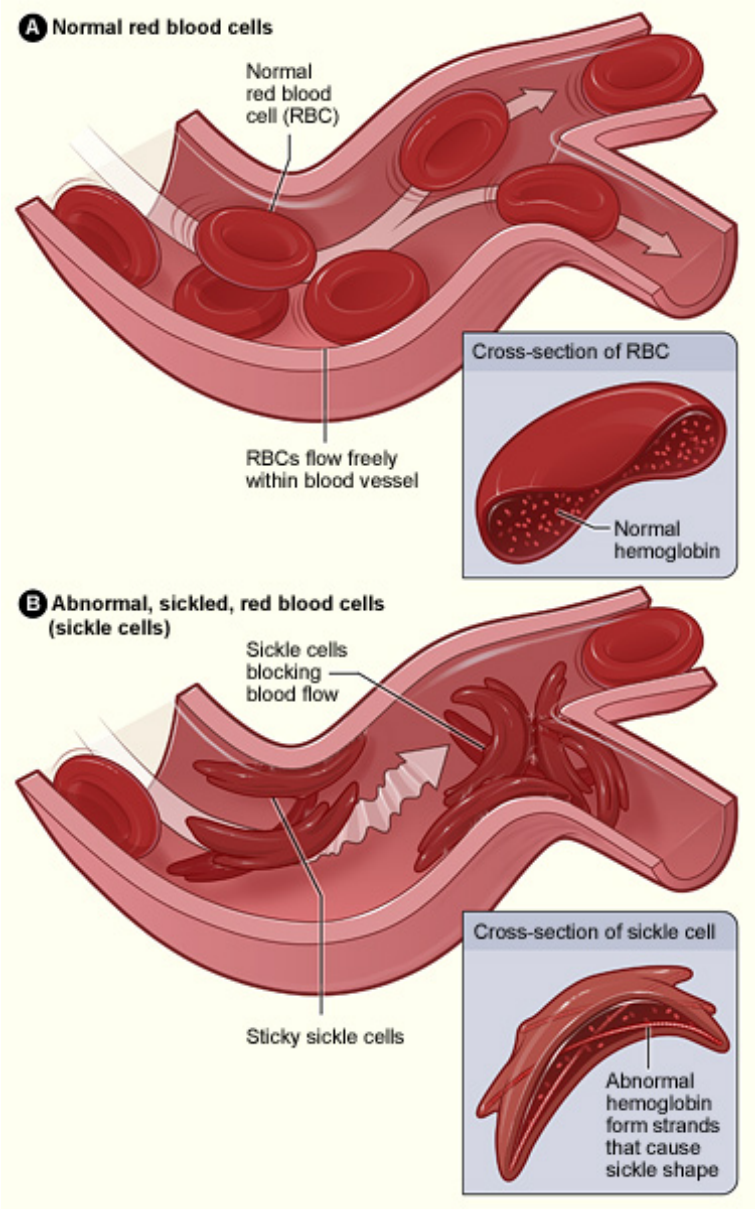






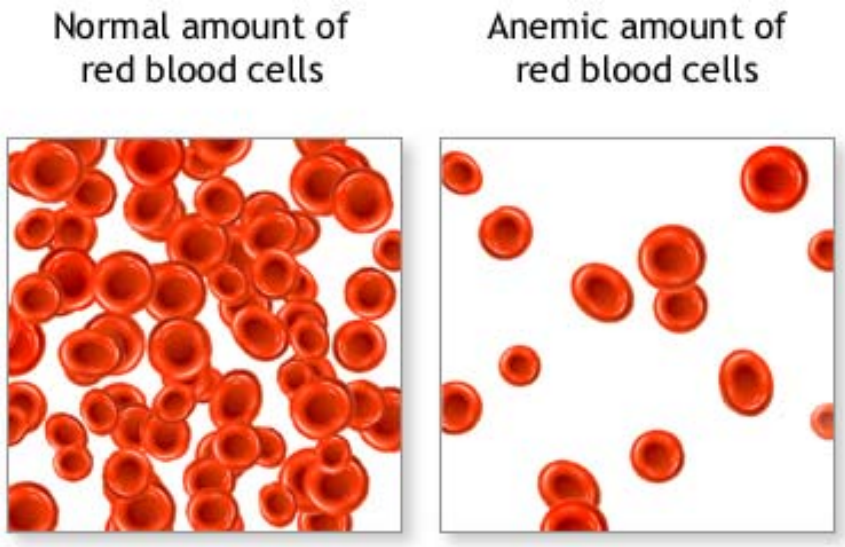
Spherocytosis is caused by mutations of proteins of the cytoskeleton: spectrin, ankyrin band 3 or proteins 4.1 and 4.2





Also in sickle cell anhemia the reduced elasticity of RBCs leads to hemolysis

The procedure will be presented in this experiment is not commonly used in clinical practice, because it takes time and does not allow the study of large numbers of patients: simple investigations such as the reticulocyte count (immature red blood cells) can give indications on the presence of anemia hemolytic and are cheaper (but less direct).



Mesurement of the osmotic shock resistance of RBC

Materials:
RBC from a healthy donor.
Physiological salt solution NaCl = 0.15M = 0.9% w/v
Centrifuge tubes
Centrifuge
Spectrophotometer

Procedure:
Calculate the osmolarity of (C1) NaCl (OsM)

$$[NaCl] = 0.15M\ [1+\alpha(\nu-1)] = 300\ OsM$$

Table 1: Fill in the table with the table using the formula: C1xV1=C2xV2.
N.B.: Before calculating the volume of H_2O to be added, subtract the volume of cells.

	1	**2**	**3**		
	RBC	ml of NaCl (concentrated solution) V1	ml of H_2O	Final volume V2	Final osmolarity OsM C2
A	0.05ml	1.95 ml	0 ml	2 ml	0.3 OsM
B	0.05ml	ml	ml	2 ml	0.2 OsM
C	0.05 ml	ml	ml	2 ml	0.16 OsM
D	0.05ml	ml	ml	2 ml	0.15 OsM
E	0.05ml	ml	ml	2 ml	0.1 OsM
F	0.05ml	ml	ml	2 ml	0 OsM

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	1	**2**	**3**		
	RBC	ml of NaCl (concentrated solution) V1	ml of H_2O	Finale volume V2	Final osmolarity OsM C2
A	0.05ml	1.95 ml	0	2 ml	0.3 OsM
B	0.05ml	1.33 ml	0.62 ml	2 ml	0.2 OsM
C	0.05 ml	1.06	0.88 ml	2 ml	0.16 OsM
D	0.05ml	1 ml	0.95 ml	2 ml	0.15 OsM
E	0.05ml	0.8 ml	1.15 ml	2 ml	0.12 OsM
F	0.05ml	0.67 ml	1.28 ml	2 ml	0.1 OsM
G	0.05ml	0 ml	1.95 ml	2 ml	0 OsM

Spectrophotometer

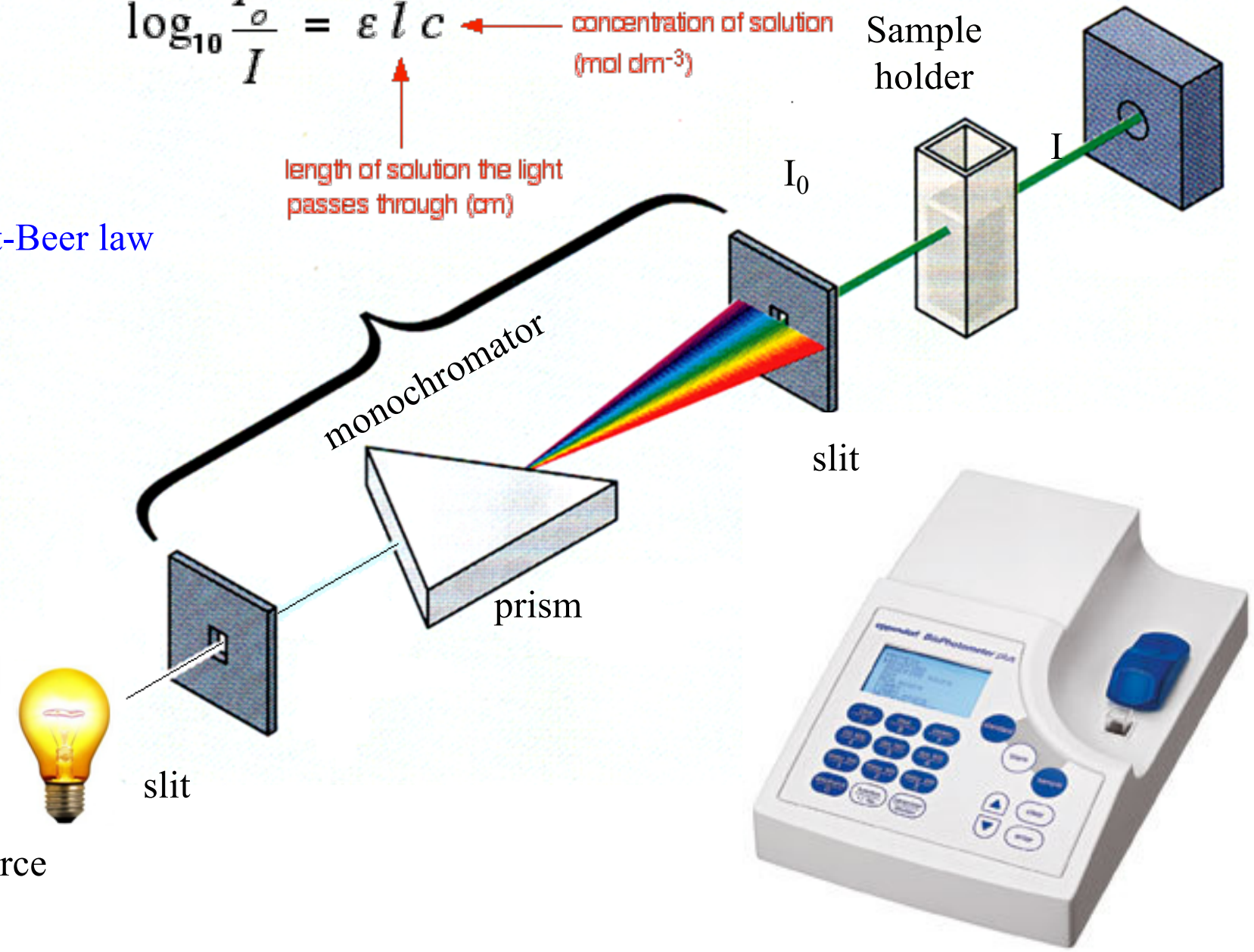
Greek letter, epsilon

$$\log_{10}\frac{I_o}{I} = \varepsilon\, l\, c$$

concentration of solution (mol dm^{-3})

length of solution the light passes through (cm)

Lambert-Beer law



Fill the tubes with the amounts indicated in columns 1, 2, 3.
Spin for 1 minute at 5000rpm.
Collect the tubes.
Transfer the supernatant into a cuvette and measure the optical absorbance.

Table 2 – measure the optical density at (OD) 560nm convert them into lysis percentage %, using this formula: OD(F) : 100% = OD(x) : X

	Concentration of NaCl	OD at 560 nm	% lysis
A	0.3 OsM		0%
B	0.2 OsM		
C	0.16 OsM		
D	0.15 OsM		
E	0.1 OsM		
F	0 OsM		100%

Absorption spectra of HbA

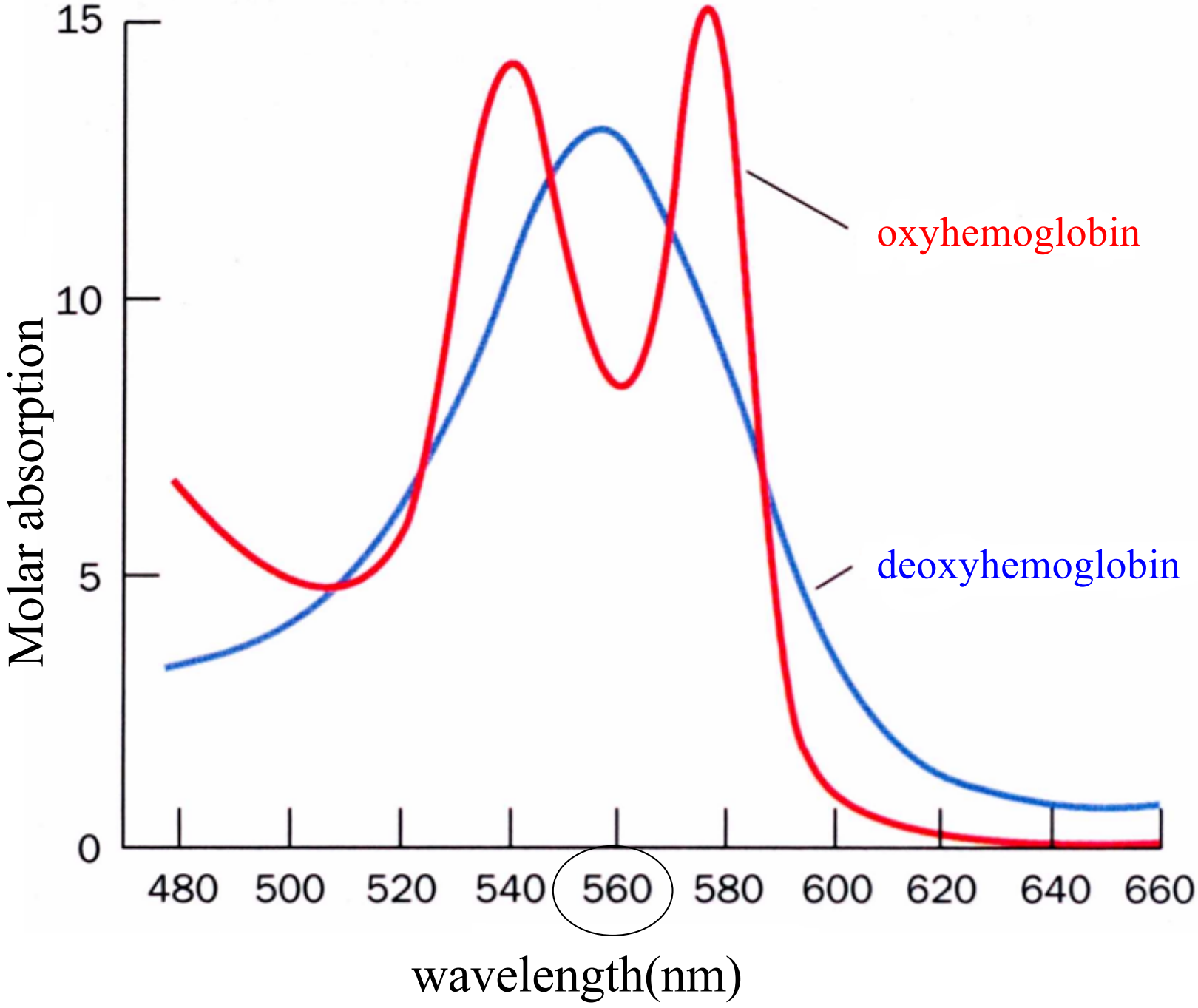


Table 2

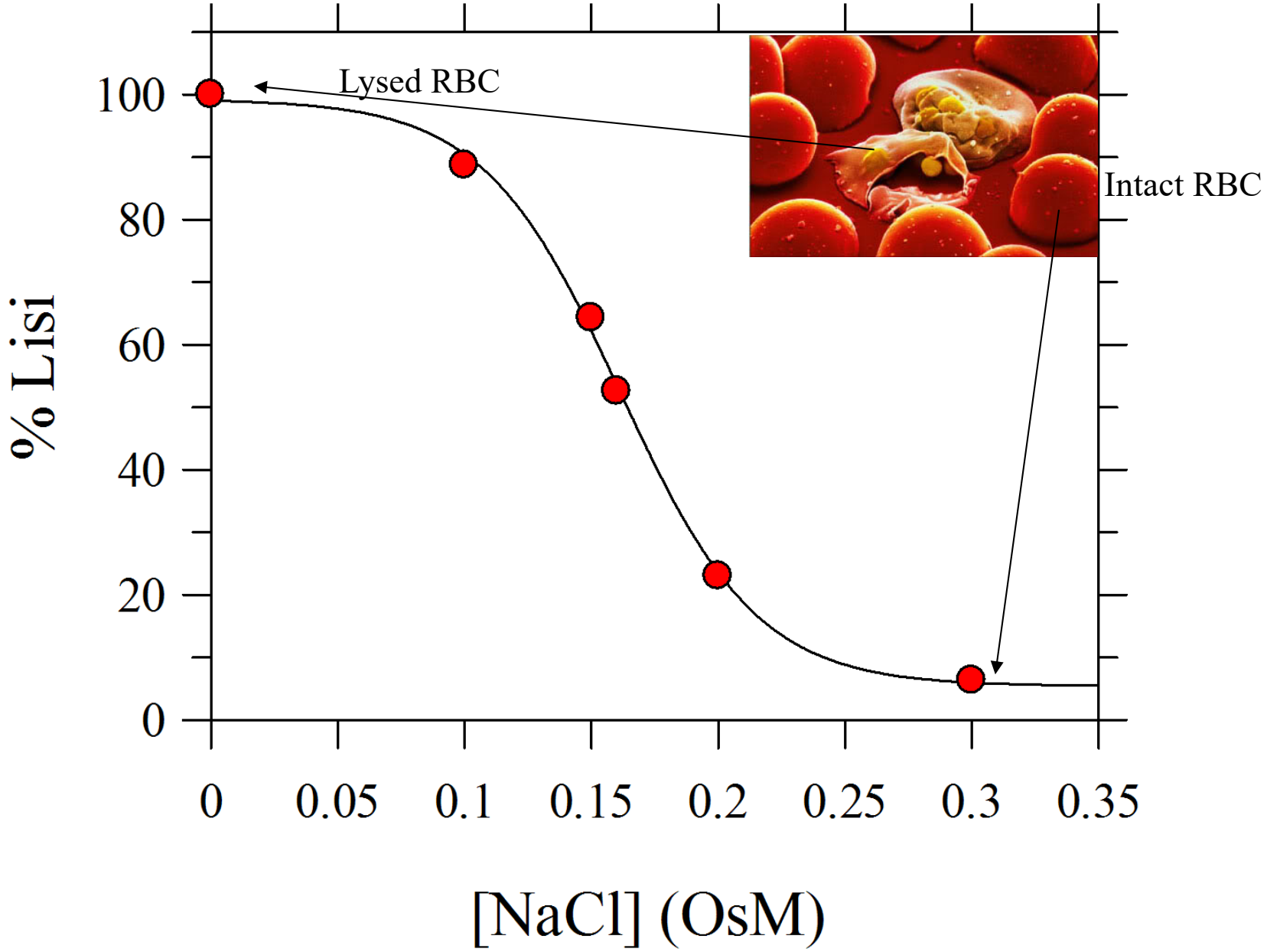
	Concentration osM NaCl (x axis)	OD at 560 nm	% lysis (y axis)
A	0.3 osM		
B	0.2		
C	0.16		
D	0.15		
E	0.1		
F	0		100%

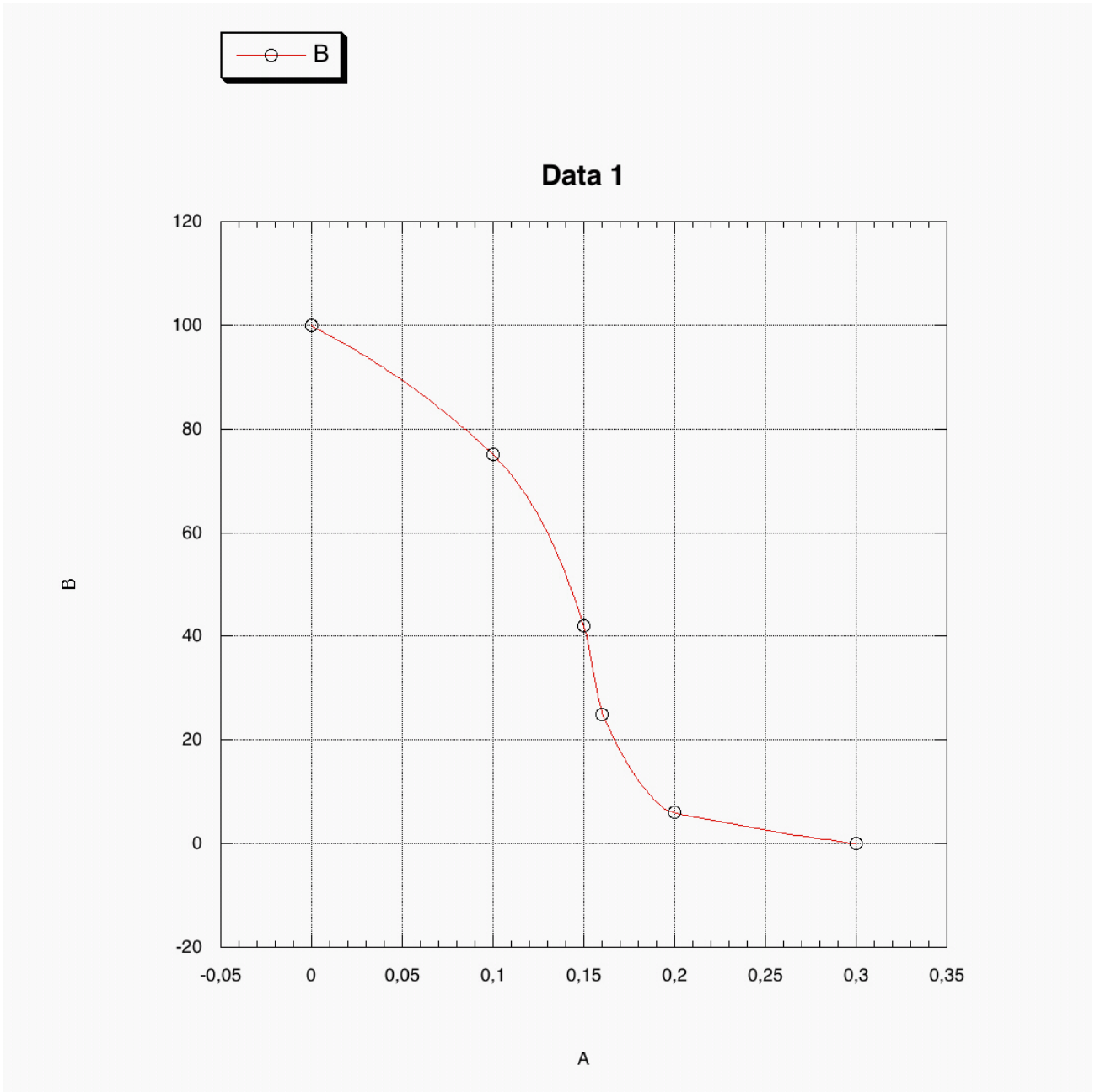
Hemolysis fraction calculation: $(OD_F\text{-}OD_A):100 = OD_i:x$

$$x \quad \frac{100 \quad OD_i}{(OD_F \quad OD_A)}$$

The correctin is needed because RBC are not synchronized and also at isotonic conditions there is a small amount of lysis (physiologic levels between 1 and 5%)

The data from table 2 should yield a sigmoid curve




B
Data 1
120
100
80
60
40
20
0
-20
B
-0,05
0
0,05
0,1
0,15
0,2
0,25
0,3
0,35
A