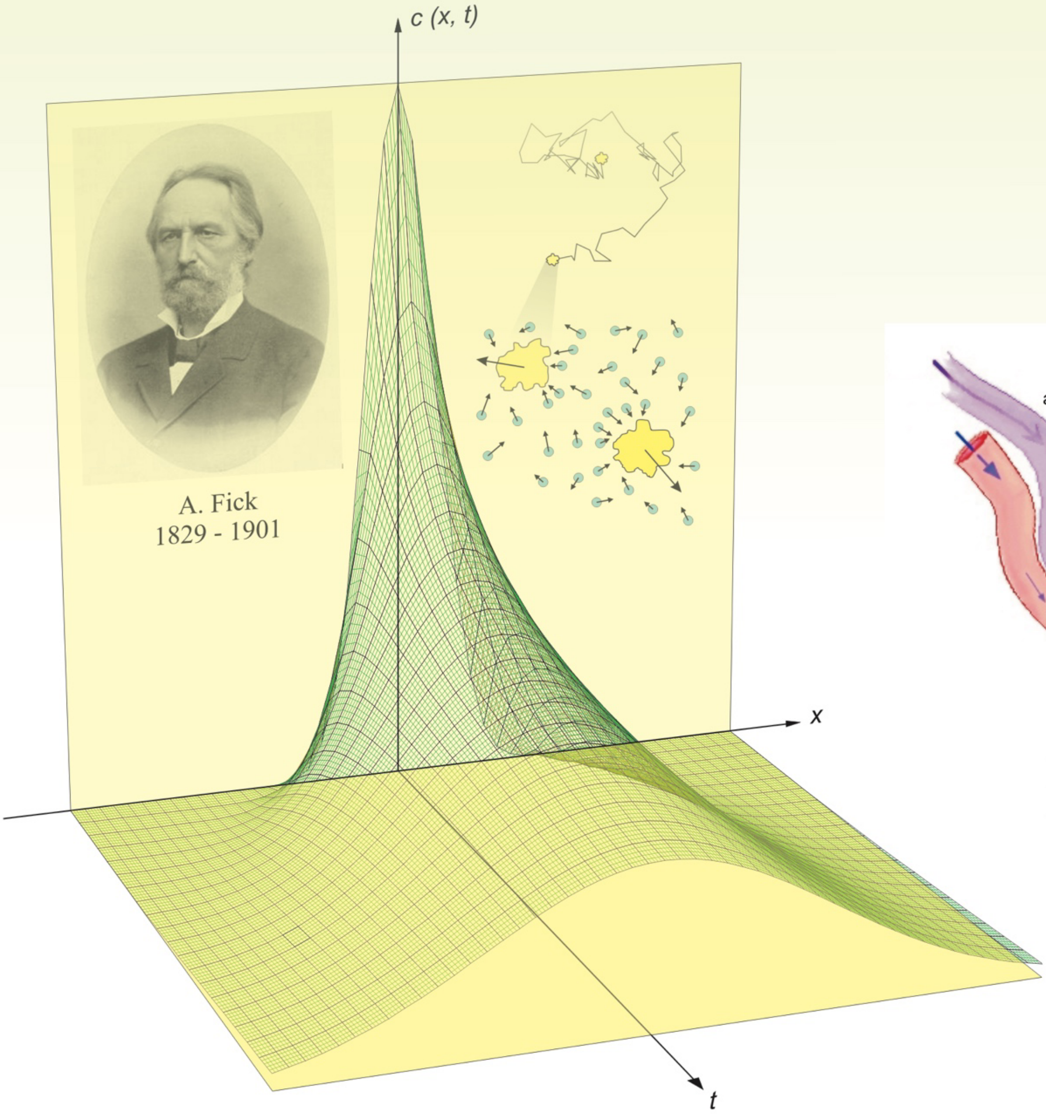
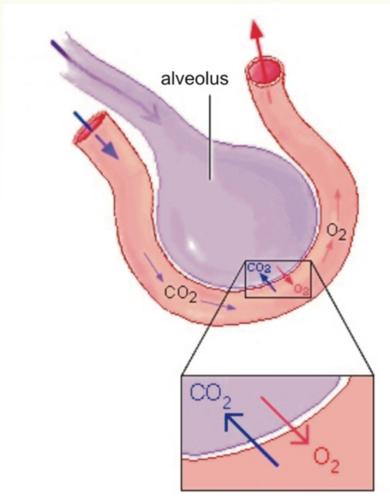
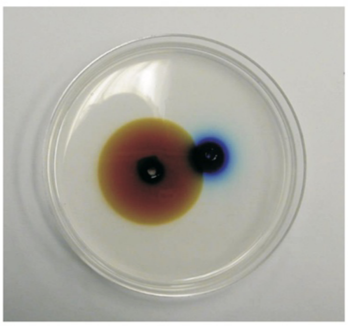
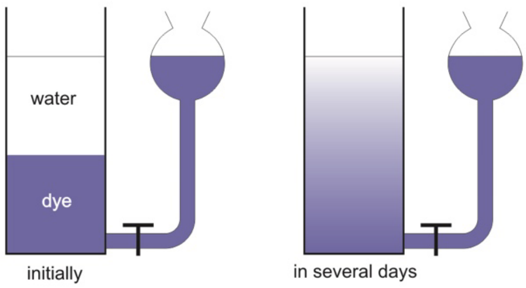
DIFFUSION

MATERIAL TRANSPORT, DETERMINATION OF THE DIFFUSION COEFFICIENT









SUMMARY:

BROWNIAN MOTION: Brown first observed the irregular, random movement of pollen grains mixed with water during microscopic examination of the pollen suspension. This observable movement is the apparent consequence of the continuous collisions of the restlessly moving invisible molecules driven by thermal energy.

DIFFUSION: The same invisible thermal motion results in diffusion, which can be observed macroscopically. If you drop some ink droplets into a glass of water, it slowly spreads and after a while it will stain the whole liquid, without any external intervention. This process of spreading is called diffusion, which, in thermal equilibrium, continues until the distribution of particles becomes uniform in the volume. The Brownian motion of the particles continues, but is no longer macroscopically detectable due to the uniform particle distribution resulting from the equilibrium.

MATTER FLOW DENSITY or FLUX (J_v): Simply, it expresses the "strength" of diffusion, or in other words the physical quantity characterising the spread of a diffusing substance: it is the number of moles of substance per unit area per unit time that pass through as a result of diffusion; its unit is mol/(m^2·s).

CONCENTRATION DROP: The $\Delta c/\Delta x$ quantity that expresses the rate of change of concentration that decreases with location. Simply put, it corresponds to the difference in concentration per unit distance. (More commonly known as the concentration gradient.)

DIFFUSION COFFITIENT (D): It is a factor of proportionality, which gives the amount of substance diffused over a unit area in a unit time, if the concentration drop was also a unit; its unit is m^2/s. Its value depends on temperature, viscosity of the medium, particle size and shape.

FICK'S FIRST LAW: the flow of particles per unit time across a unit area (flux) is proportional to the concentration drop, i.e. $J_v = -D \cdot \frac{\Delta c}{\Delta x}$, where coefficient D is the diffusion coefficient.

FICK'S SECOND LAW: describes the spatial and temporal changes of the concentration as

$$D \cdot \Delta t \frac{\Delta\left(\frac{\Delta c}{\Delta x}\right)}{\Delta x} + c_t = c_{t+\Delta t},$$

if the spatial distribution of the concentration is known at a given time [c(x,t)], it gives the distribution at a later time $t+\Delta t$.

Spreading of the particles due to the arbitrary thermal motion is known as diffusion. Sugar spreads in the coffee (even without stirring) or the rose scent spreads in the room, both by diffusion. This process goes on (it is noticeable) in case of thermal equilibrium until the distribution of the particles becomes even in the entire volume. Diffusion is extremely important in the living organism. For example, the exchange of oxygen and carbon dioxide between the air in the alveoli of the lungs and the blood within the pulmonary capillaries, and later between the blood and the cells occurs by diffusion. Water, as a very small molecule passes the cell membrane by diffusion as well. In this practice we will learn the laws of diffusion and we will perform a measurement of a characteristic parameter of diffusion.

Further readings:
Damjanovich-Fidy-Szöllősi:
III /2.1.

diffusion
Diffusion
diffúzió

THEORETICAL OVERVIEW

FICK'S LAWS

The main question according to the diffusion process is what parameters determine the "strength" of diffusion. To characterize this, we define the matter flow density (also called in general: **flux**):

$$J_\upsilon = \frac{\Delta\upsilon}{\Delta t \cdot \Delta A}, \tag{1}$$

that gives the amount of chemical material ($\Delta\upsilon$) [*delta ˈnjuː*] that passes through a unit area (ΔA) in unit time (Δt). Its unit is mol /(m^2·s). The answer to the previous question is given by Fick's first law (for stationary diffusion), which can be given in its simplest form as:

$$J_\upsilon = -D \cdot \frac{\Delta c}{\Delta x}, \tag{2}$$

diffusion coefficient
Diffusionskoeffizient
diffúziós együttható

where $\Delta c/\Delta x$ is the concentration change along a unit distance (along the x axis), or the **concentration drop** (also known as **concentration gradient)**. Thus, the flow density of particles per second is proportional to the concentration drop (see Fig. 1). The coefficient of proportionality D is called the diffusion coefficient, which gives the amount of material that diffused through a unit area, in a unit time driven by a unit concentration drop. The unit of the diffusion coefficient is m^2/s. The diffusion coefficient depends on the size and shape of the diffusing particle and on the viscosity and temperature of the medium (see Table 1). For spherical particles the diffusion coefficient can be calculated from the Einstein-Stokes formula as:

$$D = \frac{kT}{6\pi\eta r}, \tag{3}$$

where r is the radius of the particle, η is the viscosity and T is the thermodynamic temperature of the medium.

diffusing particle (molecular weight)	medium	D (m^2/s)
H_2 (2)	air	$6.4\cdot10^{-5}$
O_2 (32)	air	$2\cdot10^{-5}$
CO_2 (44)	air	$1.8\cdot10^{-5}$
H_2O (18)	water	$2.2\cdot10^{-9}$
O_2 (32)	water	$1.9\cdot10^{-9}$
Glycine (75)	water	$0.9\cdot10^{-9}$
Serum albumine (69 000)	water	$6\cdot10^{-11}$
Tropomyosine (93 000)	water	$2.2\cdot10^{-11}$
Tobacco mosaic virus (40 000 000)	water	$4.6\cdot10^{-12}$

***Table 1.** Diffusion coefficients of some substances at 20 ºC.*

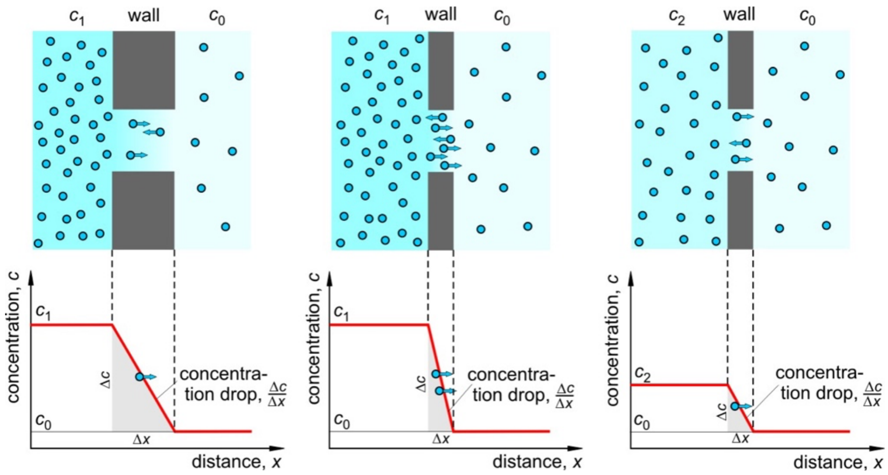


***Fig. 1.** Demonstration of Fick's first law. The concentration drop (Δc/Δx) determines the "strength" of the diffusion in a given system. In the left and central panels, the concentration differences are identical but across different distances. In the central and right panels, the concentration differences are different across the same distance. Thus, in the left and right panels the identical concentration gradients drive diffusion of the same flux.*

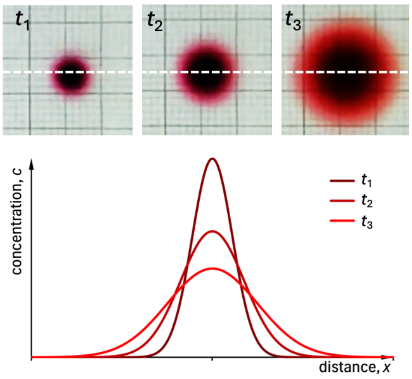


***Fig. 2.** Diffusion of potassium permanganate in agar gel. **Top:** The spot width increases with time due to diffusion. **Bottom:** concentration profiles along the dashed lines drawn in the center of the spots are normally distributed at each time point.*

> ***The normal (Gaussian) distribution***
>
> The concentration distributions shown in Figure 2 can be mathematically approximated by the normal or Gaussian distribution. The bell-shaped curve has one peak, and its edges extend to infinity in both directions. The shape of the curve is determined by two parameters, **the expected value, denoted by μ, and the standard deviation, denoted by σ.**
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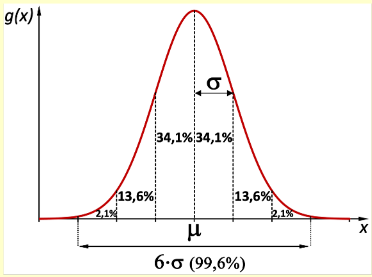
> 
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>
> The area under the bell curve can be divided according to the distance from the expected value. Although the curve is theoretically infinitely wide, 99.6% of its area in both directions falls within the range of $\mu \pm 3\sigma$. It follows from this, that the width of the K-permanganate spots in Figure 2 is approximately $6 \cdot \sigma$.

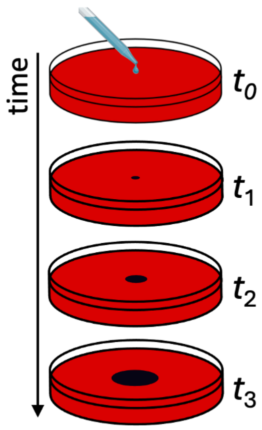


***Fig. 3.** The diffusion of protons in agar gel stained with Congo red indicator can be tracked through a color change.*

Another important question about diffusion is how fast the process takes place, e.g. an equilibration of concentrations. Fick's 1st Law does not take into account the possible variation of concentration over time, so in practice it can be applied to cases where the concentration drop is considered constant over time. An example is the diffusion of respiratory gases between the alveolar lumen and the capillary, because respiration and circulation maintain a constant concentration drop between the two sides of the respiratory membrane. It can also be used when the observation is of such short duration that the change in concentration is negligible. However, it cannot be used directly if the change in concentration over time is significant. Fick's 2nd Law describes this, namely the spatio-temporal variation of concentration:

$$D \cdot \Delta t \frac{\Delta\left(\frac{\Delta c}{\Delta x}\right)}{\Delta x} + c_t = c_{t+\Delta t} \,. \tag{4}$$

This relationship tells us that if we know the (spatial) distribution of the concentration at a given time t, then what the new concentration distribution will be at a slightly later time of $t + \Delta t$. As an example, we show in Fig. 2 the diffusion of K-permanganate starting from a point in agar gel. As time progresses, the K-permanganate spot spreads wider and wider in the gel, which can be characterized by the concentration distributions shown in the figure.

DETERMINATION OF THE DIFFUSION COEFFICIENT

In this experiment, we use Fick's Second Law to determine the diffusion coefficient of H^+ ions. To begin, we apply a drop of hydrochloric acid to the center of a thin agar gel disk. From this initial point, we track the diffusion of free protons by observing the color change of the Congo red indicator mixed into the gel. As the protons diffuse, they lower the pH, causing the indicator to shift from red to dark blue. Although the concentration distribution shown in Figure 2 is not directly visible, the position of the diffusion front can be accurately determined (Figure 3). By measuring the diameter of the dark blue spot at regular intervals, we can quantitatively monitor the diffusion process. When we plot these measured diameters against time, we obtain the following result:

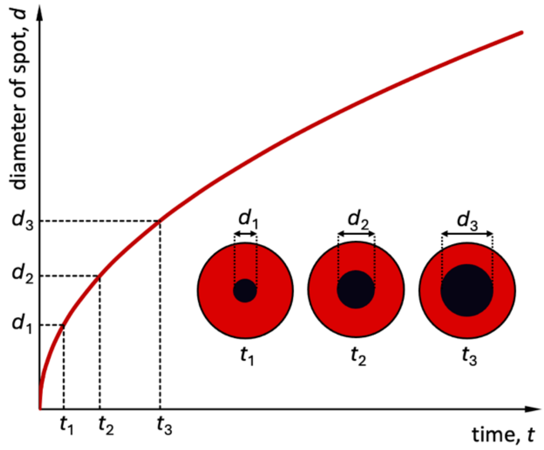


***Fig. 4.** Diffusion of H^+ ions in agar gel stained with Congo red indicator. The increase in the diameter of the dark spot follows a square root function over time.*

From the graph in Figure 4, we observe that the spot diameter (d) increases at a decreasing rate over time, following a square root function. This indicates that the rate of diffusion slows as time progresses. The concentration distribution along the diameter of the expanding spot can be approximated by a normal distribution (Figure 5). The width of this distribution, represented by the standard deviation (σ), is determined by the diffusion coefficient (D) and the elapsed time (t) according to the following relationship:

$$\sigma = \sqrt{2Dt} \,. \tag{5}$$

Since the pH of the gel stays low enough to keep the indicator dark within three standard deviations from the center in both directions, we define the spot's diameter as $6 \cdot \sigma$ in our evaluation (Figure 5).

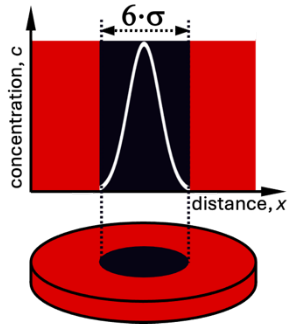


Fig. 5. The relationship between the concentration distribution of H^+ ions and the observed color reaction in the gel. The width of the dark spot can be well approximated by six times the standard deviation of the Gaussian curve describing the concentration distribution.

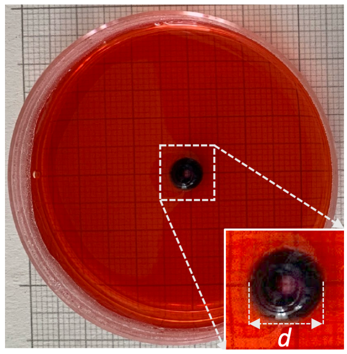


Fig. 6. *During the experiment, we measure the spot's diameter using millimeter paper placed beneath the gel.*

Based on this, the temporal change in the diameter of the spot can be described by the following equation:

$$d = 6 \cdot \sqrt{2Dt}\ , \tag{6}$$

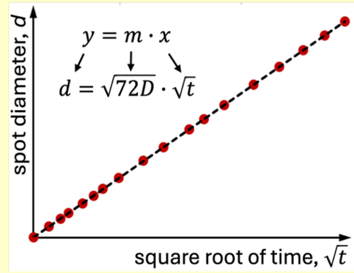
here d is the diameter of the spot, t is the diffusion time, and D is the diffusion coefficient. From this, we can determine the diffusion coefficient of free H^+ ions in the agar gel. If we plot the change in the spot diameter as a function of the square root of time, we obtain a straight line according to the following equation:

$$d = \sqrt{72D} \cdot \sqrt{t}\ , \tag{7}$$

a linear equation where the independent variable is $\sqrt{t}$, the dependent variable is d, and the slope is $\sqrt{72D}$. By plotting the spot diameters against the square root of time and determining the slope, the diffusion coefficient can be easily calculated as

$$D = \frac{slope^2}{72}. \tag{8}$$

Plotting the measured data
According to Equation (7), the spot diameter is proportional to the square root of time. Therefore, the most straightforward way to visualize the data is by plotting the measured spot diameters against the square root of the elapsed time. This results in a straight line, where the slope (m) is related to the square root of the diffusion coefficient. By determining the slope, we can calculate the diffusion coefficient using Equation (8).



TASKS:

Using a micropipette, carefully dispense 5 µl of 1M HCl into the small indentation at the center of the Congo red-stained agar gel. The indicator's color change will be visible immediately. Start the stopwatch as soon as the solution is added. At the specified time points in the protocol, measure the spot's diameter using the millimeter paper placed beneath the gel.

1. Place the Petri dish containing the Congo red-stained agar gel disk on millimeter paper, ensuring that the indentation in the center of the gel aligns with a clearly identifiable coordinate.
2. Using a micropipette, dispense 5 µl of 1M HCl into the indentation at the center of the gel. As soon as the solution is added, have your partner start the stopwatch.
3. At the specified time points in the Excel protocol, measure the diameter of the spot.
   If possible, take a photo of the gel with your phone at each time point. This will allow you to zoom in on the image for more precise measurements (Figure 6).
4. Calculate the square root of the elapsed time (in seconds) and convert the spot diameter to meters.
5. Create a scatter plot of the spot diameter (in meters) as a function of the square root of time (in seconds).
6. Fit a straight line to the data points, then calculate the diffusion coefficient using the slope of the fitted line and Equation (8).
7. Using the obtained diffusion coefficient to calculate the time required for different spot diameters to form by using Equation (7).