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Laboratory Experiment

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Exploring Drug Diffusion through a Membrane: A Physical Chemistry Experiment for Health and Life Sciences Undergraduate Students

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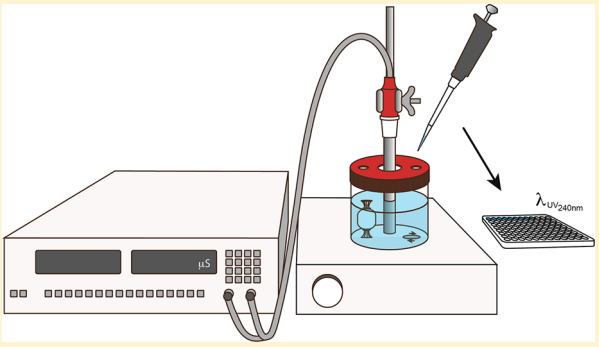
Supporting Information

ABSTRACT: The transport of molecules across biological membranes are critical for most cellular processes. Membrane permeability is also a key determinant for drug absorption, distribution, and elimination. Diffusion, that is, the migration of matter down a concentration gradient, is a simple mechanism by which both endogenous and drug molecules can enter (or exit) cells. This paper describes a simple and engaging physical chemistry undergraduate laboratory experiment for health and life sciences students studying diffusion of drug and dye molecules in solution. In this experiment, the diffusion of amitriptyline hydrochloride, ranitidine hydrochloride, and tartrazine across a cellulose dialysis membrane is evaluated using an innovative macro and microscale approach. Compound concentration in solution as a function of time is obtained both from conductivity measurements of 25 mL solutions and from absorbance measurements of 100 μL samples taken in a 96-well microplate. By using solutions of different concentrations, the permeability coefficient of the membrane and the diffusion coefficient of the tested compounds are determined. Comparison of both methods, which yield similar values, is performed, which shows that either one of the approaches is suitable to independently conduct the experiment. On the basis of diffusion phenomena, the important pharmaceutical issue of absorption and transport of drugs through passive diffusion across biological or artificial membranes is presented.



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Molecular diffusion is an important phenomenon in processes such as dissolution and transport through biological or synthetic membranes, for example, polymer membranes used for controlled-release drug delivery systems.[1−5] Cell membranes act as selective barriers between the interior of cells and their external environment.

Molecules in solution diffuse spontaneously from high concentration to low concentration regions until uniformity in solution composition is attained; thus, concentration gradient is the driving force for the diffusion process. This molecular transport, in solution or across a barrier, is currently expressed as flux, that is, the rate of diffusion of molecules across a plane per unit area. Flux is proportional to the concentration gradient, and the proportional constant is the diffusion coefficient according to Fick's first law:[1−4]

$$J = -D\left(\frac{\partial c}{\partial x}\right) \tag{1}$$

where J is the flux of a component across a plane of unit area, $(\partial c/\partial x)$ is the concentration gradient, D is the diffusion coefficient, and the minus sign indicates that flux is in the direction of decreasing concentration. In SI units, J is expressed in mol m^{-2} s^{-1}, c in mol m^{-3}, and ∂x in m; therefore, the units of D are m^2 s^{-1}.

Experimentally, it is easier to measure changes in concentration as a function of time, that is, $(\partial c/\partial t)$ rather than $(\partial c/\partial x)$, which for molecular diffusion across a membrane, is related with the flux J according to[1−4]

$$J = \frac{V}{A}\left(\frac{\partial c}{\partial t}\right) \tag{2}$$

where A is the area of the membrane, V is the volume of solution of concentration c, and t is time.

At steady-state conditions (constant flux), Fick's first law is simplified by[3,4]

$$J = D\frac{(c_1 - c_2)}{l} = P\Delta c \tag{3}$$

where l is membrane thickness, P is the permeability coefficient, and c_1 and c_2 are the concentrations of the drug substance in the donor and receiving compartments, respectively. Perme-

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ability coefficients obtained in appropriate cell models are valuable tools for the prediction of drug bioavailability.[3,4]

For experiments under sink conditions, $c_2 = 0$ and $c_1 = \Delta c$, where c_1 is the initial concentration of the solution. Thus, the slope of the plot of J against c, which corresponds to the permeability coefficient, allows determination of the diffusion coefficient once membrane thickness is known.[3,4]

The current laboratory experiment is based on the determination of the diffusion coefficients of the tricyclic antidepressant drug amitriptyline hydrochloride (AMT), the histamine H_2-receptor antagonist ranitidine hydrochloride (RNT), and the FDA-approved food dye tartrazine (TTZ) (Figure 1), across a cellulose membrane, using solutions of

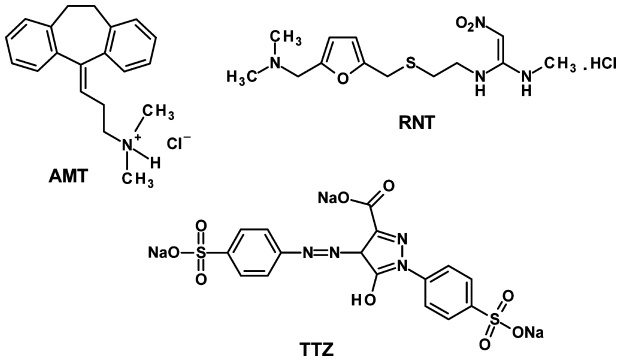

Figure 1. Chemical structures of amitriptyline hydrochloride (AMT), ranitidine hydrochloride (RNT), and tartrazine (TTZ).

different concentrations contained inside a dialysis bag immersed in water. A model cell is thus simulated by the dialysis membrane, and the effect of different concentration gradients on the rate of diffusion is assayed. The permeability coefficient and the diffusion coefficient of the tested compounds are then obtained from plots of flux against concentration.

Some undergraduate experiments have been described in the chemical education literature that involve diffusion of a solute,[6−10] either in free solution or across a membrane, and determination of diffusion coefficients has been made by different techniques such as fluorescence spectroscopy,[6] pulsed-field gradient NMR,[7] capillary electrophoresis,[8] and cyclic voltammetry.[9] In the present experiment, a new approach is proposed: electrical conductance (macroscale procedure) and UV absorbance (microscale procedure) to monitor diffusing agent concentration in solution as a function of time. Students are introduced to these two different physical methods and compare both methodologies to demonstrate their suitability. Moreover, since AMT is a controlled substance and a hazardous drug, students are taught laboratory safety measures regarding the use and disposal of toxic chemicals in a controlled, low to moderate risk.

The experiment has been typically performed by a maximum of 16 health sciences students enrolled in the second-year undergraduate physical chemistry course offered by the Faculty of Pharmacy at the University of Lisbon (ULisboa). The entire laboratory procedure and statistical data analysis can be completed in a single 3 h period by students working in pairs. Each group of students studies a different compound, and all data are gathered at the end of the experiment. Students are then encouraged to suggest factors that can account for the different values obtained for each compound.

MATERIALS AND METHODS

AMT, RNT, and TTZ were purchased from Sigma (St. Louis, MO). Students were provided with a 100 mmol L^{-1} AMT stock solution and 10 mmol L^{-1} stock solutions of RNT and TTZ, prepared by accurately weighting the powders. For the diffusion assays, standard solutions were prepared by dilution of the stock solutions. Standard solutions were also diluted in order to construct a calibration curve from conductivity and absorbance measurements, as described in the Supporting Information. All solutions were prepared with deionized water (Milli-Q water purification system) and yielded conductivity values always lower than 2 μS cm^{-1}.

A 5 m length high grade regenerated cellulose dialysis membrane (Cellu Sep H1), with 40 mm flat width and 28 μm wall thickness and a molecular weight cutoff (MWCO) of 8000, was used for the diffusion assays. Rectangular membrane pieces of approximately 8 cm length were cut and used to prepare a dialysis bag, which was filled with 2.5 mL of the standard solution of the desired concentration and sealed. Special care was taken to avoid air bubbles inside the dialysis bag.

The dialysis bag was immersed in 25 mL of deionized water contained in a glass beaker under magnetic stirring. Conductance measurements were taken every 30 s for 10 min, while 50 μL aliquots of the aqueous solution were simultaneously collected at 1 min intervals. Appropriate dilution of the collected samples was performed, then 100 μL of the diluted solutions was transferred to a 96-well UV-microplate, and absorbance measurements at maximum wavelength of the tested compound were carried out in a Spectrostar Omega plate reader.

Conductivity data were obtained from conductance measurements collected at 1 kHz with a Wayne-Kerr B905 automatic precision bridge (WKR, England) at room temperature using a Ingold conductivity cell type 980-K19/120 with platinum electrodes and a cell constant of 1.15 cm^{-1}.

HAZARDS

AMT is harmful if swallowed and causes serious eye irritation. AMT is also associated with reproductive toxicity. Students must wear protective clothes, nitrile gloves, and safety goggles to manage AMT solutions. Hands must be carefully washed after glove removal to avoid accidental ingestion. AMT is very toxic to aquatic life[11] with long-lasting effects. Therefore, discharge into the environment must be strictly avoided, and all safety measures regarding waste disposal are mandatory. AMT residues should be disposed in a closed container available at the laboratory and sent to an approved waste disposal plant.

RNT is not a hazardous drug and is not classified as a dangerous substance. The stock solution of TTZ provided has a concentration in the range of the acceptable daily intake of up to 7.5 mg/kg of body weight per day.

RESULTS AND DISCUSSION

Diffusion of AMT, RNT, and TTZ across a cellulose dialysis membrane was studied by simultaneously taking conductivity and absorbance readings of the surrounding aqueous solution as a function of time for different concentrations inside the dialysis bag. Thus, influence of different concentration gradients on the diffusion rate across the model cell membrane was assayed. Concentration of the diffusing agent was obtained from conductivity and absorbance calibration curves and plotted against time for both methods.

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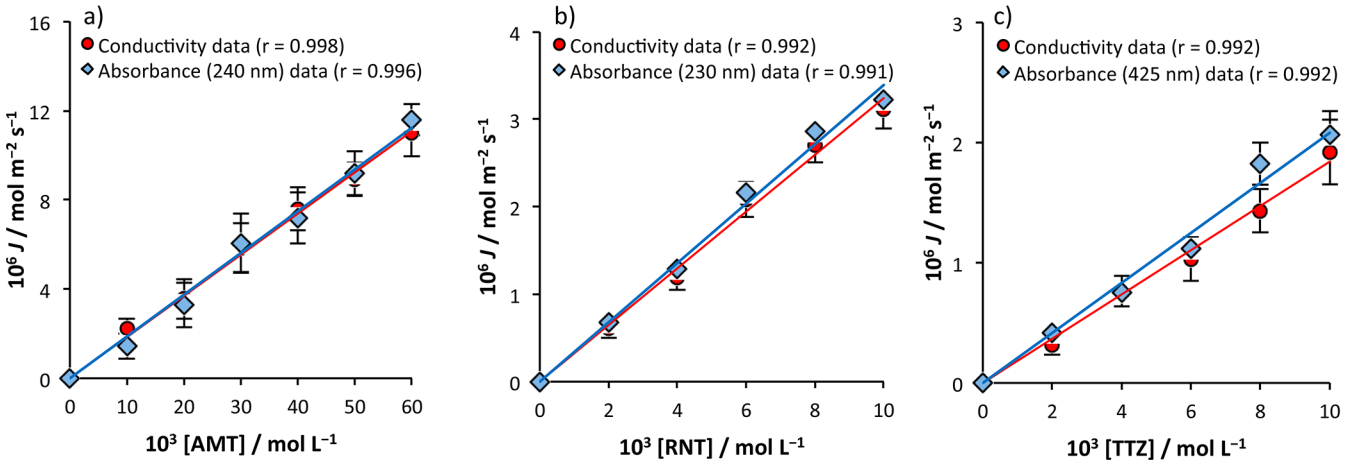

Figure 2. Plots of flux versus concentration for (a) amitriptyline hydrochloride (AMT), (b) ranitidine hydrochloride (RNT), and (c) tartrazine (TTZ).

Table 1. Permeability Coefficient (P) and Diffusion Coefficient (D) of AMT, RNT, and TTZ Across a Cellulose Dialysis Membrane, At Room Temperature

compound	AMT		RNT		TTZ	
method	10^7 P/m s^{-1}	10^{12} D/m^2 s^{-1}	10^7 P/m s^{-1}	10^{12} D/m^2 s^{-1}	10^7 P/m s^{-1}	10^{12} D/m^2 s^{-1}
conductivity	1.85 ± 0.29	5.18 ± 0.82	3.24 ± 0.27	9.07 ± 0.76	1.94 ± 0.25	5.43 ± 0.70
UV Abs	1.87 ± 0.32	5.24 ± 0.90	3.45 ± 0.15	9.66 ± 0.42	2.08 ± 0.10	5.82 ± 0.80
mean	1.86 ± 0.31	5.21 ± 0.86	3.35 ± 0.21	9.37 ± 0.59	2.01 ± 0.18	5.63 ± 0.75

Diffusion rates were obtained from the slopes of the plots of concentration as a function of time, which correspond to the concentration gradient, and the flux J was determined according to eq 2 since the area of the dialysis bag is known. Plots of J against total compound concentration obtained by students in a typical laboratory experiment are shown in Figure 2.

Higher concentration gradients yielded higher diffusion rates, as expected, with increase in flux linearly as concentration increased (Figure 2). The slopes of these plots correspond to the permeability coefficient, P, assuming steady-state conditions, where eq 3 can be applied.[3,4] The diffusion coefficient across the cell model was then determined since membrane thickness is known. Representative mean experimental values are presented in Table 1. Similar values were obtained from either conductivity or absorbance data, which suggest that both methods are reliable for diffusion studies of the tested compounds in the concentration ranges employed. Moreover, depending on the available equipment, the instructor may select either the macro or the microscale approach. If there is some equipment constrains or if it is not possible to simultaneously measure the conductance of several solutions (e.g., computer-controlled acquisition of data), the mentioned micro method is an excellent alternative.

Higher values of 2.46×10^{-10}, 8.24×10^{-10}, and 4.9×10^{-10} m^2 s^{-1} have been reported in the literature[12−14] for the diffusion coefficients of AMT, RNT, and TTZ, respectively, in aqueous media at room temperature. In the present experiment, the cellulose membrane was found to slow down the diffusion of the studied compounds as compared to diffusion in pure water, that is, in the absence of a barrier, which resulted in a lower diffusion coefficient. Although the cellulose membrane used, with a MWCO of 8000, is permeable to the tested molecules, the diffusing molecules still have to hit the membrane pores in order to be able to pass to the surrounding solution. Thus, the presence of the membrane influences diffusion rate and consequently the diffusion coefficient.

CONCLUSION

In this paper, we report a new laboratory experiment that introduces the important phenomenon of diffusion across a membrane using a model cell consisting of a dialysis bag made up of a cellulose membrane containing solutions of AMT, RNT, and TTZ at different concentrations. Both macroscale (conductivity measurements) and microscale (absorbance measurements) procedures were used to measure the variation of drug and dye concentration in the surrounding solution as a function of time. The results obtained allowed the determination of the diffusion coefficient of the tested compounds across the cellulose membrane, and both methods yielded similar consistent values proving their suitability to accomplish the study. This simple laboratory experiment was planned to be performed by health sciences students at the undergraduate level and engages the students by presenting the important pharmaceutical issue of drug absorption through passive diffusion across biological or artificial membranes.[1−5]

ASSOCIATED CONTENT

Supporting Information

Student handouts and instructor notes, including CAS number of chemicals and safety warnings. This material is available via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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