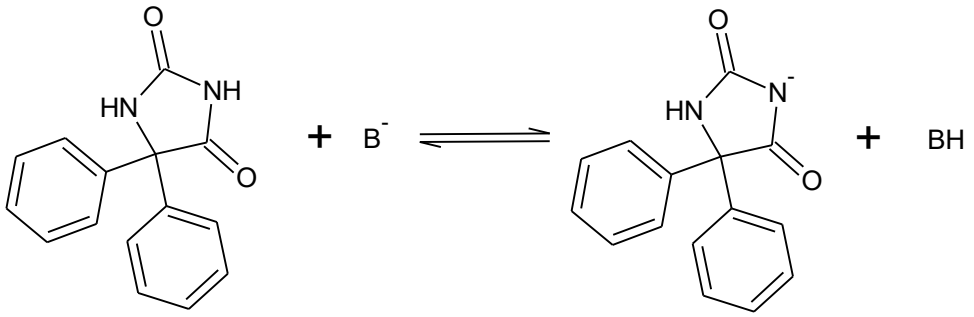
6. Determination of the acid dissociation constant of phenytoin



M.W. = 252.27

Method principle

Dissociation of weak acids including phenytoin, i.e. 5,5-diphenylimidazolidin-2,4-dione, an antiepilepsy drug, can be expressed by Henderson-Hasselbach equation, which can be written in the form

$pK_a = pH + \log([HA]/[A^-])$, [1]

where **[HA]** is the equilibrium concentration of non-dissociated form of the acid and **[A^-]** is the equilibrium concentration of its dissociated form.

Spectrophotometric determination of dissociation constant requires significant changes of absorption spectrum in dependence on pH. The optimal wavelength which is needed to be chosen is that, which gives the maximum difference of absorbances measured in strongly acid and strongly alkaline solutions respectively, i.e. between absorbances of fully dissociated and fully non-dissociated forms of a compound. Thus we will measure the absorbance of the compound in its fully dissociated form (in this task in solution of NaOH of concentration 0.01 $mol.l^{-1}$) and consequently in the form in which dissociation is completely suppressed (here in solution of HCl of concentration 0.01 $mol.l^{-1}$). For absorbance of a substance, the Lambert-Beer's law is valid

$A = \varepsilon.c.l$, [2]

where **A** is absorbance, **ε** is molar absorptivity, and **l** is length of the layer through which the light goes (i.e. cuvette width). Absorbance of phenytoin in fully dissociated form, i.e. in solution of a strong base, can be expressed

$A_{A^-} = \varepsilon_{A^-}.[A^-].l = \varepsilon_{A^-}.c_N.l$, [3]

where $\mathbf{c_N}$ is total concentration of phenytoin, and, because all the substance is under these conditions

dissociated, $\mathbf{[A^-] = c_N}$. Absorbance of phenytoin in fully non-dissociated form can be expressed similarly

$A_{HA} = \varepsilon_{HA} . [HA] . l = \varepsilon_{HA} . c_N . l ,$ [4]

because all the substance is in non-dissociated form which means $[HA] = c_N$.

Further we will determine absorbances of the set of phenytoin solutions with pH graded so that the substance will be almost completely in form HA in the most acid one and completely in form A^- in the most alkaline one. The range of pH values could be chosen so that the numeric value of pH which corresponds with assumed or estimated value of pK_a, which is to be determined, was set in the middle of this range. *Such estimation of pK_a value can be based on analogy, or can be performed by calculation by means of a suitable software.* The particular absorbance of every of the set of nitrofurantoin solutions can be expressed by the equation

$A = \varepsilon_{A^-} . [A^-] . l + \varepsilon_{HA} . [HA] . l$ [5]

The resolution of equations [2] - [5] gives for the ratio of equilibrium concentration in investigated solutions $[HA] / [A^-]$

$[HA] / [A^-] = (A_A - A) / (A - A_{HA})$ [6]

The substitution the formula [6] for $[HA]/[A^-]$ in equation [1] results in the equation

$pK_a = pH - \log \{(A_A - A) / (A - A_{HA})\}$ [7]

which must be enumerated for every buffered solution. The final value of pK_a is then acquired as the mean (= the average) of all the partial values.

Procedure

1.Preparation of solutions

The set of stock buffer solutions of concentration 0.2 $mol.l^{-1}$ and pH 7.8, 8.0, 8.2, 8.4, 8.6, 8.8 and 9.0 is diluted with distilled water into the set of buffer solutions of concentration 0.01 $mol.l^{-1}$. The stock solutions of hydrochloric acid and sodium hydroxide respectively are diluted into the solutions of the concentration 0.01 $mol.l^{-1}$ too. 100.00 ml of the stock solution of phenytoin of concentration 0.005 $mol.l^{-1}$ in ethanol is prepared in a suitable volumetric flask. 0.5 ml of the phenytoin stock solution is then pipetted into every of the set of nine 50 ml volumetric flasks. The flasks are then filled up to the mark with the appropriate diluted solution of hydrochloric acid, buffer or sodium hydroxide respectively. The set of comparison solutions (blanks) is prepared similarly but 0.5 ml of pure ethanol is pipetted into every volumetric flask instead of the phenytoin stock solution.

2a. Measurement on a simple Jenway 6305 UV-VIS spectrophotometer

Switch the instrument on by the switch on its back side and leave all its tests to pass. Switch the computer, log-in into Windows and run 63-Zero software. Switching-on and setting-up of both spectrophotometer and its controlling computer are performed by a lecturer or a laboratory assistant. Select *Concentration-Method* option, input the wavelength 236 nm and confirm *Update instrument.* Insert the cuvette filled with the appropiate blank solution and in the option *Measure* click to *Cal to blank. (If any number is in the window Auto-read, delete it.)* Wash the cuvette repeatedly with a small volume of the sample and then fill the cuvette with this sample. Measure the absorbance by clicking *Read.* **Write down the absorbance immediately.** (Printing of results is difficult at this instrument).The pairs blank-sample must be measured in the ascending order of pH values. This means that the solutions in hydrochloric acid are measured first, the buffered solutions pairs follow in the order from the lowest pH to the highest one and the solutions in sodium hydroxide are the last. The order must be kept to minimize the error of measurement caused by mixing of a measured solution with the rest of previous one. *Measured values are possible to clear by closing and new opening of **63-Zero** software only.*

2b. Measurement on a Hewlett-Packard 8453 UV-VIS spectrophotometer

*Switching-on and setting-up of both spectrophotometer and its controlling computer are performed by a lecturer or a laboratory assistant. We measure absorption spectra in the ultraviolet region in range from 220 to 320 nm. We determine absorbances at the wavelength of **236 nm**.*

Procedure of the proper measurement

Only one cuvette (cell) is used for whole measurement. In order to minimize errors caused by changes of solutions in it, it is recommended measure the particular samples together with their blanks in order of increasing or decreasing pH, so that the measurement starts with blank and sample containing hydrochloric acid, continues with those of pH 7.8, 8.0, 8.2, 8.4, 8.6 and 9.0 and finally blank and sample with sodium hydroxide are measured. Or contrariwise, the measurement can start with solutions with hydroxide, continue with buffers of pH from 9.0 to 7.8 and finish with solutions with hydrochloric acid.

1. The cuvette is at least twice poured with a comparison solution (blank), then it is filled with this solution and placed into the cuvette holder of the spectrophotometer. The cuvette is fixed by the lever at the side of the holder. The spectrum of a blank is then measured by click to the button *Blank*. The blank spectrum appears in a window entitled *Lasts blank spectrum.* A sample spectrum is measured similarly, with a cuvette at least twice washed and then filled with a sample solution, by clicking *Sample* button. The window *Sample*

information appears and it is needed to write HCl or NaOH or pH of the particular buffer into the field *Sample information* in the window *Sample information* and confirm *OK*. Thus absorbances of all 8 solutions are measured. All the acquired spectra together with a table of absorbances are printed by clicking the button *Print results.*

3. Calculation of pK_a

The substitution of particular determined values of absorbance in the equation [7] gives the partial values of pK_a. The resulting pK_a value is then calculated as the arithmetic mean of these values.

Tasks:

1. Determine pK_a of disociaciation of N-H of imidazolidinedione ring of phenytoin.
2. What will be the percentage of dissociated form of the compound in blood plasma at pH = 7.4 ?
3. What will be the percentage of dissociated form of the compound in small intestine at pH = 8.0 ?
4. What will be the percentage of dissociated form in stomach at pH = 1.0 ?