SPECTROPHOTOMETRIC DETERMINATION OF THE DISSOCIATION CONSTANT OF *p*-NITROPHENOL

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Task 13

SPECTROPHOTOMETRIC DETERMINATION OF THE DISSOCIATION CONSTANT OF *p*-NITROPHENOL

I. Aim of the task

The aim of the task is determination of the dissociation constant of the weak acid whose anion absorbs light in another spectrum range than its undissociated form and to examine the dependence between the dissociation degree values and the solvent pH .

II. Introduction

1. Dissociation of electrolytes.
2. Constant and degree of dissociations.
3. Absorption of electromagnetic radiation by solutions.
4. Lambert – Beer law.

References:

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III. Theory

III.1. Dissociation of electrolytes

Dissociation is the term used to describe the breakdown of a molecule into simpler parts. This often (almost always in basic chemistry) occurs in water. The equilibrium constant for such a reaction is called a dissociation constant. The dissociation constant of a compound is important for a number of reasons:

- The dissociation constant of a compound can help us determine the environmental impact of a chemical, e.g., the potential of the chemical to adsorb to soil;
- The dissociation constant can also help us to determine the biological impact of a chemical, such as to what degree it will be absorbed by the body and the degree of toxicity. For example, ammonia, NH^+, is toxic to fish, whereas its ionized form, ammonium, NH_4^+ is not toxic;
- Dissociation constants are also useful in helping us to calculate the pH of a weak acid or base.

In aqueous solutions p-nitrophenol dissociates according to the reaction:

$$HA \rightarrow H^+ + A^-$$

Undissociated molecules of p-nitrophenol are colourless and their anions are yellow.
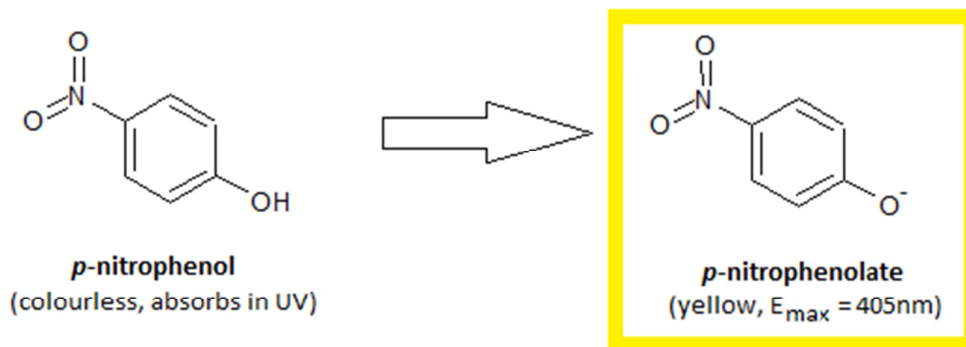

Fig. 1. Dissociation of p-nitrophenol.

Because of that undissociated acid absorbs the electromagnetic radiation in a different spectrum from that of its anion. For example, p-nitrophenol (acid form) has the maximum absorbance at approximately 320 nm and p-nitrophenolate (basic form) absorbs best at 405 nm, as shown in Figure 2.
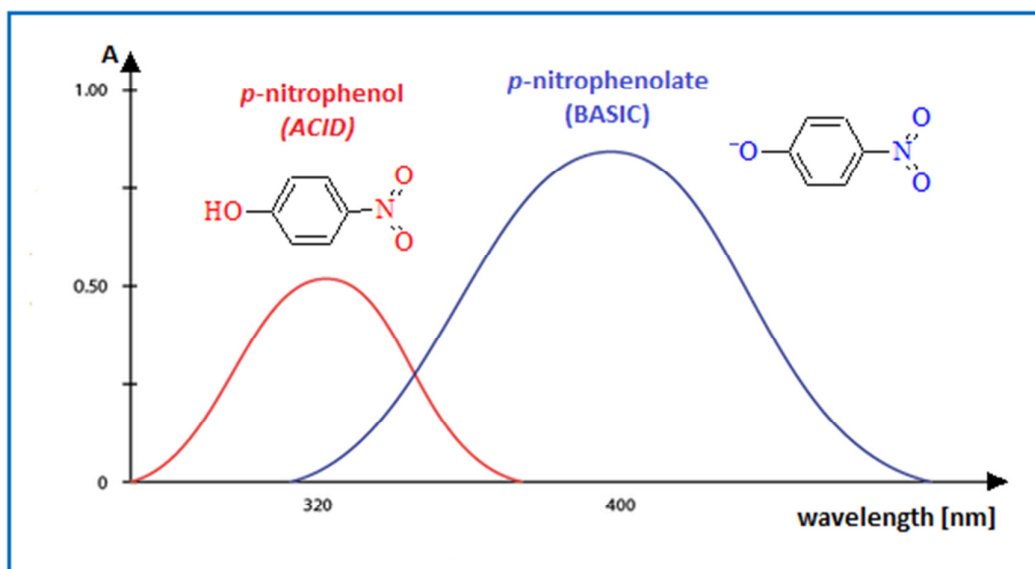
Fig. 2. Absorbance of two different compounds: *p*-nitrophenol and its anion.

III. 2. Constants and degree of dissociation

The expression for the equilibrium constant has the form:

$$K = \frac{[H^+][A^-]}{[HA]} \tag{1}$$

where [HA] is the equilibrium concentration of the acid, [A⁻] its conjugate base respectively and [H⁺] the concentration of hydrogen ions in the equilibrium state.

The acid dissociation constant *K* provides information about how readily a weak acid reacts with water and other bases, allowing comparison of the reactivity (proton-donating ability) of different acids. It also provides a measure of how much the acid dissociates as the solution is made acidic or alkaline. Historically, this information has often been presented in the form of the degree of dissociation *α*, which is the fraction of the weak acid present in its dissociated, conjugate base form:

$$\alpha = \frac{[A^-]}{c_0} \tag{2}$$

where c_0 is the total, analytical concentration of the weak acid.
Obviously we can also define α as the fraction of the weak acid that is not dissociated. Therefore:

$$[A^-] = \alpha \cdot c_0 \tag{3}$$

and

$$[HA] = c_0 - \alpha \cdot c_0 = (1 - \alpha) \cdot c_0 \tag{4}$$

Taking into account equations (3) and(4), we can express **the dissociation constant** by the equation:

$$K = \frac{[H^+]\alpha}{1-\alpha} \tag{5}$$

The connection between the constant and the degree of dissociation (equation 5) can be expressed as follows:

$$\frac{1-\alpha}{\alpha} = \frac{1}{K}[H^+] \tag{6}$$

Because K is a constant, the expression $(1-\alpha)/\alpha$ is a linear function of concentration of hydrogen ions:

$$y = ax \tag{6a}$$

where $y = \dfrac{1-\alpha}{\alpha}$, $x = [H^+]$ and the coefficient $\dfrac{1}{K}$ is the slope of the line.

To find the equilibrium constant for the ionization of a weak acid we must know the concentration of hydrogen ions and their corresponding values of the dissociation degree (α).

If we use buffer as a solvent of p-nitrophenol solutions, then we will know the value of pH and can calculate concentration of hydrogen ions. Undissociated molecules of p-nitrophenol are colourless and their anions are yellow. Thus, each of them absorbs light in a different range of spectrum. This creates favourable conditions for the determination of the degree of dissociation of p-nitrophenol by the use of photometric method. Phenolate ions more strongly absorb light at the wavelengths $\lambda = 455$nm. Then we can pass over the absorption of the undissociated p-nitrophenol form. Therefore if substances comply with the Lambert-Beer law, the absorbance of the solution depending on the concentration of phenol ionsis:

$$A = \varepsilon_{A^-} \cdot [A^-]d \tag{7}$$

where ε_{A^-} is the molar absorption coefficient and d is the thickness ofthe light absorbing layer of solution.

If we assume that p-nitrophenol in the NaOH solution is completely dissociated, $[A^-]$ is equal to the total p-nitrophenol concentration, absorbance in such a solution is equal to A_o:

$$A_0 = \varepsilon_{A^-} \cdot c_0 \cdot d \tag{8}$$

If we use equations (2), (7), (8) we get:

$$\alpha = \frac{A}{A_0} \tag{9}$$

III. 3. The absorption of electromagnetic radiation by solution

There are numerous methods for measuring the concentrations of specific substances within solutions. One commonly-used method is called **spectrophotometer** (or colorimeter). All substances in solution absorb light of a wavelength and transmit light of other wave-

lengths. **Absorbance** is a characteristic of a substance just like melting point, boiling point, solubility or density. Absorbance can be related to the amount of the substance in solution, thus it can be used to quantitatively determine the amount of substance that is present.

To understand the processes in chemistry that generate light, we must realize first that light has a **dual nature**. Sometimes it behaves like a particle (called a photon), which explains how light travels in straight lines. On the other hand it behaves like a wave, which explains how light bends (or diffracts) around an object.
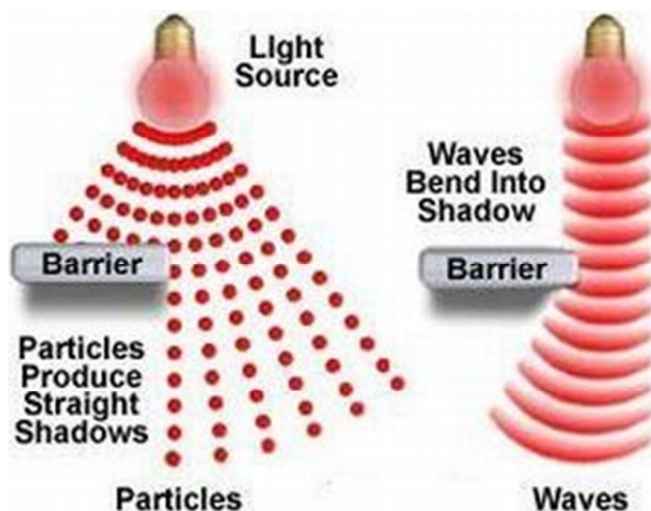

Fig. 3. The dual nature of light [5].

Physicists classify light waves by their energies (wavelengths). The wavelength, λ (lambda), is the distance between the adjacent wave crests. Labelled in decreasing energy, we might draw the entire electromagnetic spectrum as shown in the figure below:

Electromagnetic radiation can be divided into various regions according to the wavelength. The visible region has the wavelengths of 400-700nm. In this region, lights of different energy have different colours: violet and blue in the low wavelength region and orange and red in the high wavelength region. When a substance in solution appears blue, it means that the substance is absorbing red light and transmitting blue light. A substance which appears red absorbs blue light and transmits red light. A substance is said to have absorption spectra for a blue substance and a red substance.
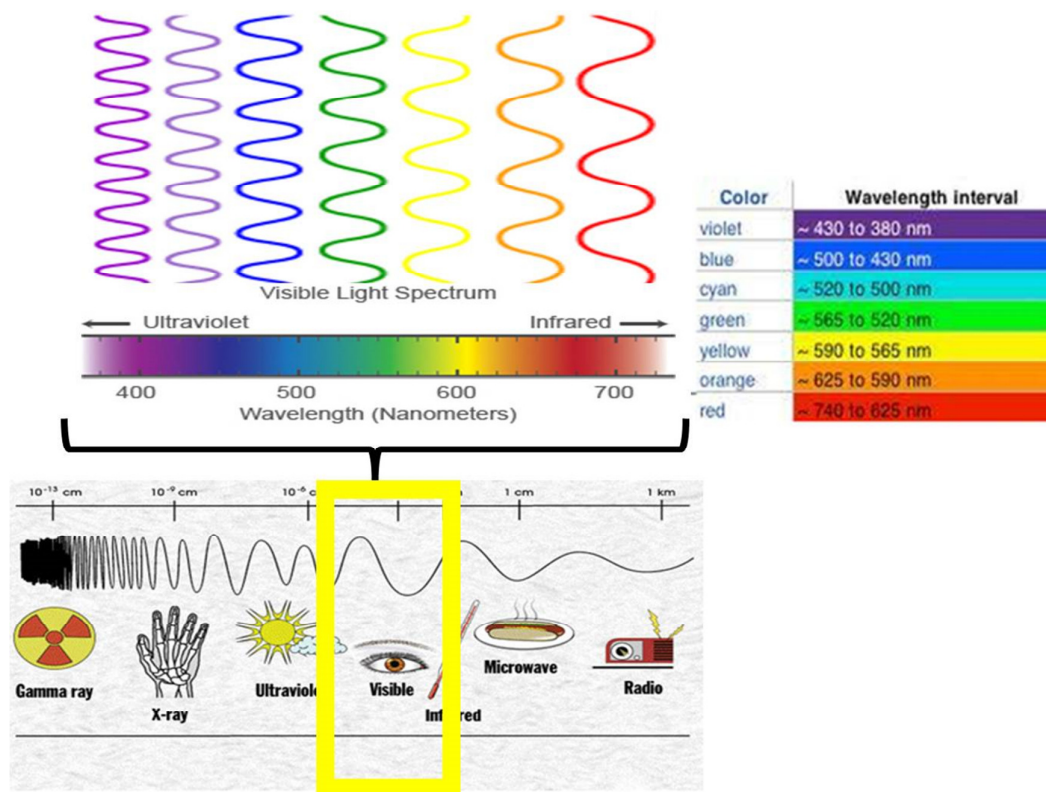
Fig. 4. The Electromagnetic Spectrum. Notice how small the visible region of the spectrum is compared to the entire range of wavelengths [6].

III. 4. Lambert-Beer law

Because there is a mathematical relationship between the solute concentration and the light absorption/transmittance, we can use a spectrophotometer to determine the concentration of a substance in a sample of any solution where we do not know yet the substance concentration by comparing its light absorbance or transmittance with that of a solution that has a known concentration of that substance (called a reference, or a standard solution).

For example, let us say we want to determine the concentration of glycerol in a sample of blood plasma. We mix the glycerol with a reagent that changes and that absorbs light at a specific wavelength when it reacts with the glycerol (this allows us to identify light absorbance by glycerol as opposed to that by other substances in the solution). We then determine the light absorbance of the solution by placing the solution in a spectrophotometer (an instrument that measures light absorbance at specific wavelengths). We determine the absorbance of our blood plasma sample. We also run the same procedure on a glycerol solution of known concentration and determine its absorbance.

We define transmittance T as the ratio of the amount of light transmitted to that of light that initially fell on the surface. This is expressed in the following formula:

$$T = \frac{I}{I_o} \tag{10}$$

where I_o is the intensity of the incident light beam and I is the intensity of the light coming out of the sample.
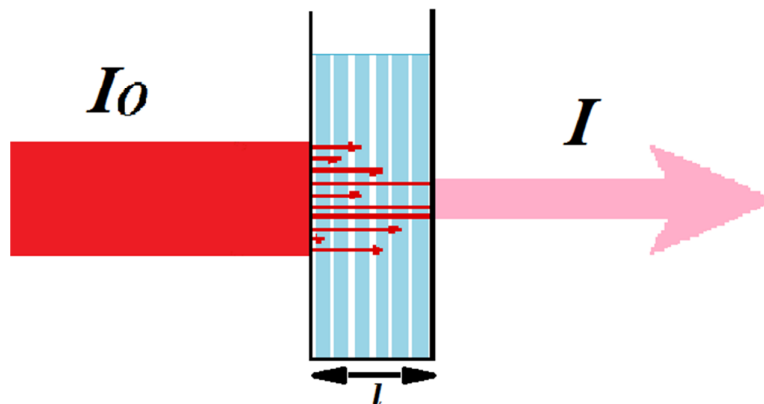

Fig. 5. Diagram of transmittance of a light beam as it travels through a cuvette of width l .

Transmittance is the relative percent of light that passes through the sample. Thus if half the light is transmitted, we can say that the solution has 50% transmittance.

$$T(\%) = \frac{I}{I_o} \times 100\% \tag{11}$$

Absorbance is related to transmittance:

$$A = -log\frac{I}{I_o} \tag{12}$$

Increasing the thickness (optical path length) of an absorbing layer by dl the transmitted intensity decreases by dI. Therefore this derivative

$$-\frac{dI}{dl} \tag{13}$$

is negative, and directly proportional to the material coefficient ε, the total transmitted intensity, I and the concentration of light absorbing material in the solution, of c concentration.

$$-\frac{dI}{dl} = I \cdot \varepsilon \cdot c \tag{14}$$

Integrating this equation between limits of the incoming (I_0) and transmitted (I) intensities

$$\int_{I_0}^{I} -\frac{1}{I}dI = \varepsilon \cdot c \int_{0}^{l} dl \tag{15}$$

the definite integral

$$-\left(\ln I - \ln I_0\right) = \varepsilon \cdot c \cdot l \tag{16}$$

Rearranging and introducing the wavelength dependence

$$\ln \frac{I_0}{I} = A = \varepsilon \cdot c \cdot l \tag{17}$$

$$A(\lambda) = \varepsilon(\lambda) \cdot c \cdot l \tag{18}$$

where: A is a dimensionless number, ε is the proportionality constant (called **the molar extinction coefficient** or **the molar absorptivity**). It is a constant for a given substance, provided the temperature and wavelength are constant. It has units of $[dm^3/mol]$, c is a concentration $[mol/dm^3]$, l is a path length of the cuvette containing the sample.

The absorbance is linearly dependent on the concentration of absorbing material and the optical path length, l. When the concentration is given in $[mol\ dm^{-3}]$ the coefficient ε is called the molar absorbance.

The usual non SI unit of thickness is cm, and the unit of ε is $[dm^3 mol^{-1} cm^{-1}]$. The absorbance and molar absorbance are wavelength or frequency dependent. Absorbance is the concentration and optical path length dependent too, but molar absorbance is not.

Deviations from the Beer–Lambert law

The Beer-Lambert Law may not be applicable to all solutions since they can ionize, polymerize at higher concentrations, or precipitate to give a turbid suspension that may increase or decrease the apparent absorbance. Furthermore, the Beer-Lambert Law is the most accurate between absorbance of **0.05 and 0.70 (max. 1).** Above 0.70, the measured value tends to underestimate the real absorbance. Below the value of absorbance equal to 0.02 many instruments are not accurate.
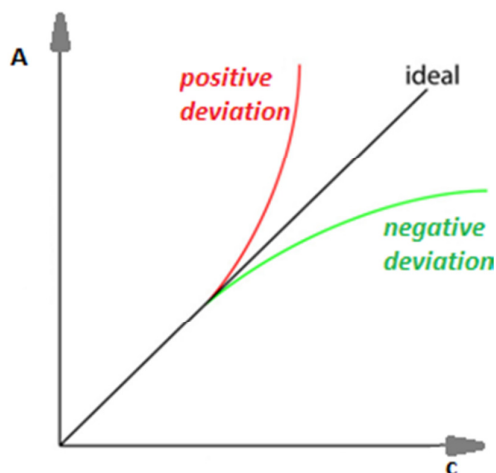

Fig. 6. Calibration curves showing positive and negative deviations from the ideal Beer's law calibration curve, which is a straight line.

Under certain conditions the Beer–Lambert law fails to maintain a linear relationship between attenuation and concentration of the analyte. These deviations are classified into **three categories:**

1. **Real**– the fundamental deviations due to the limitations of the law itself.
2. **Chemical**– the deviations observed due to specific chemical species of the sample which are being analyzed.
3. **Instrument** – the deviations which occur due to how the attenuation measurements are made.

IV. Experimental

A. Devices and materials

1. **Device:** Spectrophotometer Cecil 1011.
2. **Equipment:**
   - Erlenmeyer flask: 50 cm^3 – 6u,
   - measuring flask: 25 cm^3 – 10 u,
   - graduated pipettes: 10 cm^3 and 25 cm^3,
   - cuvette of the thickness d = 1 cm (1 x 2 x 3 cm).
3. **Materials:**
   - p-nitrophenol solution in NaOH, $5 \cdot 10^{-4}$ mol/dm^3;
   - aqueous solution of p-nitrophenol, $5 \cdot 10^{-3}$ mol/dm^3;
   - buffers: pH = 6.0; 6.5; 7.0; 7.5 and 8.0;
   - NaOH solution, 0.01 mol/dm^3

B. Program

1. Preparation of the spectrophotometer for measurements.
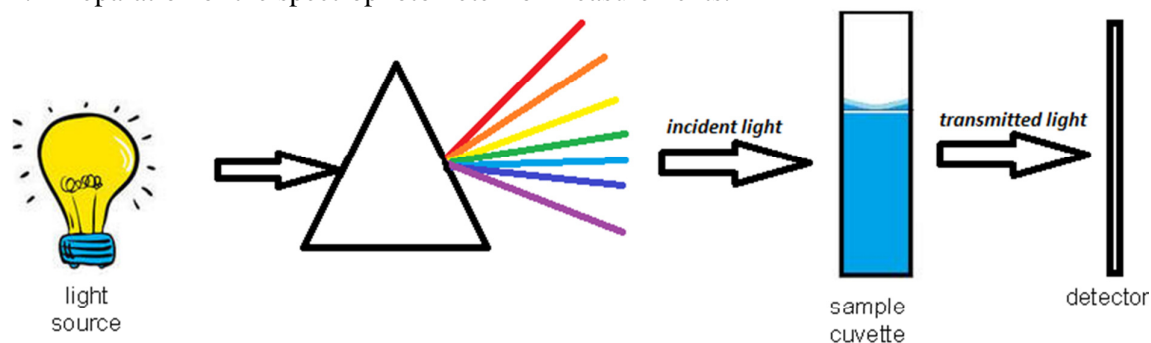

Fig. 7. Scheme of the measurement by spectrophotometer.

2. Check of the Lambert-Beer law:
   - Preparation of the p-nitrophenol solutions in 0.01 mol/dm^3 NaOH.
   - Measurement of absorption of the solutions.

3. Determination of the dissociation constant of p-nitrophenol:
   - Preparation of solutions of p-nitrophenol in various buffers.
   - Measurement of absorption of the solutions.

4. Development of the results.

C. Methods

Check the Lambert-Beer law.

The above-mentioned method of determination of the dissociation constant of *p*-nitrophenol demands using the Lambert-Beer law for the solutions of this substance. One should check if there is a linear dependence between the absorbance and the concentration of phenolan ion in a wide range of concentration.

For this purpose:

– use 25 cm^3 flasks and measure the appropriate volume of $5 \cdot 10^{-4}$ mol/dm^3 solution of *p*-nitrophenol 0.01 molar NaOH, and fill with 0.01 M NaOH solution to the mark, so as to obtain solutions of the concentration listed in Table 1. Calculate how much *p*-nitrophenol solution (cm^3) should be used to obtain the required concentrations.

Table 1.

Number of sample	Concentration of phenolan ion $c_A^- \cdot 10^{-4}$ [mol/dm^3]	Absorbance A
1	0.5	
2	1.0	
3	1.5	
4	2.0	
5	2.5	
6	3.0	
7	3.5	
8	4.0	
9	4.5	
10	5.0	

– measure and record the absorbance of each solution at 455 nm, using distilled water as a reference solution.
– put the obtained values of solutions absorbance in Table 1.

Determination of the dissociation constant of *p*-nitrophenol:

– pour 10 cm^3 of one of the buffers of pH 6.0; 6.5; 7.0; 7.5 and 8.0 into each of five Erlenmeyer flasks. Pour 10 cm^3 of 0.01 mol/dm^3 NaOH into the sixth Erlenmeyer flask. Then pour 1.0 cm^3 of an aqueous solution of *p*-nitrophenol of the concentration $5 \cdot 10^{-3}$ mol/dm^3 into each flask.
– measure and record the absorbance of each solution at 455 nm using distilled water as a reference solution.
– put the obtained values of absorbance in Table 2.

Table 2.

Number of sample	pH	Absorbance A	$[H^+] \cdot 10^6 mol/dm^3$	α	$\dfrac{1-\alpha}{\alpha}$
1	6.0				
2	6.5				
3	7.0				
4	7.5				
5	8.0				

D. Results and conclusions

1. Present the data in the form of a graph dependence between the absorbance (*A)* and the concentration of phenolan ions (c_{A^-}) based on the data in Table 1; interpret the obtained relation (examine the graph and check the Beer-Lambert law).
2. By using equation(9),calculate the degree of dissociation for all buffered solutions of *p*-nitrophenol, put the obtained values in Table 2.
3. For each solution calculate the values of the expressions$(1 - \alpha)/\alpha$ and put them inTable 2.
4. Draw the graph of the degree of *p*-nitrophenol dissociation as a function of hydrogen ions concentration and draw conclusions.

Calculate the dissociation constant (*K*) of *p*-nitrophenol using equation (6). Draw the graph of $(1 - \alpha)/\alpha$as a function of the hydrogen ions concentration. According to equation(6), this relationship is a linear function of the slope equal to$1/K$ Enter the value of the dissociation constants and check with the literature data.