

## Determination of the acidity constant of bromothymol blue

### Aims of the experiment

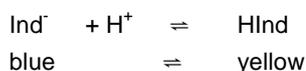
- To get to know what an acid base indicator is and how it works.
- To record and interpret absorbance spectra of dyes.
- To investigate the pH dependency of the colour of bromothymol blue.
- To determine the acidity constant and the  $pK_a$  value of bromothymol blue.
- To use and understand the Henderson-Hasselbalch equation.

### Principles

When a chemical reaction comes to a halt, an equilibrium between educt and product is reached. Depending on the position of a chemical equilibrium, small amounts of educts are still present in the reaction mixture. Furthermore, where a chemical equilibrium is concerned, we are dealing with a dynamic equilibrium. Although the proportion of product and educt in the mixture is constant, the same amount of educt continues to react to form product as product reacts back to form educt.

An acid base reaction is the prototype of an equilibrium reaction. An equilibrium is generally reached very quickly with these reactions. In a state of equilibrium, a substance is then present in both a protonated and a non-protonated form. Depending on the pH value, one form outweighs the other form. This can be investigated using acid-base indicators.

Acid-base indicators, e.g. bromothymol blue, are weak acids or bases which have different colours depending on the pH value. Different concentrations of both forms are present in a solution depending on the pH value. The protonated form is denoted  $HInd$  and the non-protonated form  $Ind^-$ . If acid is added to a bromothymol blue solution, the concentration of the protonated form  $HInd$  increases and that of the non-protonated form  $Ind^-$  decreases. The colour of bromothymol blue changes from blue (alkaline) to yellow (acidic). The equilibrium of the reaction equation:



then lies on the left side.

As in all equilibrium reactions, the law of mass action can also be employed:

$$K_s = \frac{[H^+] \cdot [Ind^-]}{[HInd]}$$

The acidity constant  $K_a$  is obtained in this way. However, in the case of acids, the  $pK_a$  value is usually stated, that is the negative decimal logarithm of the acidity constant.  $pK_a$  values are listed in tables and allow concentration-independent statements to be made about the acid strength. The pH value is linked to the  $pK_a$  value via the Henderson-Hasselbalch equation.

$$pH = pK_s + \log \frac{[\text{non - protonated form}]}{[\text{protonated form}]} = pK_s + \log \frac{[Ind^-]}{[HInd]}$$

If the relative concentrations of the protonated and the non-protonated forms are known in the equilibrium, the  $pK_a$  value can be determined.

The indicator bromothymol blue used here is blue in alkaline form and yellow in acid form. A green solution is obtained in the neutral pH area due to there being a mixture of both forms. As the absorption maxima of the two forms are far apart, the ratio of concentration of both forms can be determined at every pH value.

Absorbance spectra of bromothymol blue at various pH values are therefore recorded in this experiment. For the evaluation, the concentrations of both forms are determined using the absorbance maxima, and from this the  $pK_a$  value of bromothymol blue is calculated.



Fig. 1: Set-up of the experiment.

### Risk assessment

The substances used are in general non-hazardous. Also, it is not necessary to wear gloves when working. It is recommended, however, to wear safety glasses.

Hydrochloric acid 0.1 mol/L	
 <p>Signal word: Caution</p>	<p><b>Hazard statements</b></p> <p>H290 May be corrosive to metals.</p>
	<p><b>Precautionary statements</b></p> <p>P234 Keep only in original container.</p> <p>P390 Absorb spillage to prevent material damage.</p>

<b>Sodium hydroxide solution, 0.1 mol/L</b>	
	<b>Hazard statements:</b> H290 May be corrosive to metals. <b>Precautionary statements</b> P234 Keep only in original container. P390 Absorb spillage to prevent material damage.
<b>Signal word</b> Caution	
<b>Sodium hydrogen phosphate</b>	
	<b>Hazard statements</b> H318 Causes serious eye damage. <b>Precautionary statements</b> P280 Wear protective gloves/protective clothing/eye protection/face protection. P305+P351+P338 IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing. P313 Get medical advice/attention.
<b>Signal word:</b> Hazard	
<b>Bromothymol blue solution, 0.1 % in 20% ethanol</b>	
	Contains ethanol at a non-hazardous concentration

### Equipment and chemicals

1	Compact spectrometer USB, complete.....	467 252
2	Rectangular cuvette, glass, 10 x 10 mm.....	664 470
	better: 5 pieces, or	
5	Rectangular cuvette, plastic 10 x 10 mm.....	6640474
1	Graduated pipette, 5 mL.....	665 996
1	Pipetting ball (Peleus ball).....	666 003
5	Laboratory bottle, 100 mL, GL 45.....	602 345
2	Measuring cylinder, 100 mL.....	665 754
2	Glass rod, 200 mm x 5 mm diam.....	602 782
1	Electronic balance, 200 g: 0.01 g.....	667 7977
1	Digital pH-meter 201.....	667 4781
1	Beaker, Boro 3.3, 100 mL, hF.....	664 137
1	Wash bottle, PE, 500 mL.....	661 243
1	Bromothymol blue solution, 50 mL.....	671 0800
1	Hydrochloric acid 0.1 mol/L, 500 mL.....	674 6950
1	Sodium hydroxide solution 0.1 mol/L, 500ml.....	673 8410
1	Disodium hydrogen phosphate, 250 g.....	673 6710
1	Sodium dihydrogen phosphate, 250 g.....	673 6010
1	Buffer solution set.....	674 4600
1	Water, pure, 1L.....	675 3400
Additionally required:		
PC with Windows XP/Vista/7/8		

### Set-up and preparation of the experiment

Absorbance spectra of bromothymol blue at various pH values are recorded in the experiment. For this, buffer solutions are used having the pH values being investigated. These are mixed in the cuvette with a bromothymol blue solution and absorbance spectra are recorded.

In preparation for this, buffer solutions with sodium phosphate are initially prepared. However, other buffers with the appropriate pH values can also be used as an alternative.

#### Preparing the solutions

**Disodium hydrogen phosphate solution.** ( $\text{Na}_2\text{HPO}_4$ , 0.1 mol/L): For 100 mL of a 0.1 molar  $\text{Na}_2\text{HPO}_4$  solution, weigh out 1.42 g of disodium hydrogen phosphate and place it in a measuring

cylinder. First half-fill the measuring cylinder with water and dissolve the substance using a glass rod. Then fill the measuring cylinder up to the 100 mL mark and pour the solution into a labelled laboratory bottle.

**Sodium dihydrogen phosphate solution** ( $\text{NaH}_2\text{PO}_4$ , 0,1 mol/L): For 100 mL of a 0.1 molar  $\text{NaH}_2\text{PO}_4$  solution, weigh out 1.56 g of sodium dihydrogen phosphate and place it in a measuring cylinder. First half-fill the measuring cylinder with water and dissolve the substance using a glass rod. Then fill the measuring cylinder up to the 100 mL mark and pour the solution into a labelled laboratory bottle.

**Preparing the buffer solutions:** From both phosphate solutions, buffer solutions can now be made with various pH values, depending on how much of each of the two solutions is used. The disodium hydrogen phosphate solution acts as a base and the sodium dihydrogen phosphate solution as an acid. Buffers with pH values 6.0, 7.0 and 8.0 are required for the experiment. For this, transfer the volumes of the two solutions as shown in Table 1 (measured using the measuring cylinders) into prepared and labelled laboratory bottles:

**Tab. 1:** Preparation of the buffer solutions. Indication of the volumes of both phosphate solutions needed for the appropriate pH values.

pH value	V $\text{Na}_2\text{HPO}_4$	V $\text{NaH}_2\text{PO}_4$
6.0	12 mL	88 mL
7.0	61 mL	39 mL
8.0	95 mL	5 mL

Now check the pH values with a pH meter. Calibrate this beforehand. If the pH value of the buffer solution is too great, add the acid form of the buffer ( $\text{NaH}_2\text{PO}_4$ ). If the pH value of the buffer solution is too low, add the alkaline form ( $\text{Na}_2\text{HPO}_4$ ).

#### Construction of the experiment

Prepare the solutions (phosphate buffer, hydrochloric acid, sodium hydroxide solution and bromothymol blue solution), the graduated pipette and the cuvettes.

*Note: The experiment is easiest to perform if 5 glass cuvettes are available. The alternatives are: 5 disposable cuvettes or 2 glass cuvettes which are rinsed with distilled water between the individual measurements. It is not necessary to dry the cuvettes as the solutions are buffered.*

Connect the compact spectrometer to the computer with a USB cable. Insert the cuvette holder.

#### Measuring with SpectraLab

1. Open SpectraLab.
2. Choose the "Intensity I1" display. Start the measurement with ► (in case this does not happen automatically).
3. Switch on the lamp  on the compact spectrometer. The spectrum of the lamp is now visible. The maximum intensity should be between 75 % and 100 %. This can be adjusted via the integration time with  and . The integration time is the time during which light is collected which is then presented as the measured value. The shorter the integration time, the lower the intensity.
4. Place the black blocks (provided) into the cuvette holder to record the background spectrum (offset). Open the "Offset I0" display. The spectrum shown will be subtracted as the background spectrum during further measurements.

*Note: SpectraLab always saves the last image produced in a display. Therefore, to save the offset, for example, one only needs to leave this display. Caution: If this is accessed again during a later measurement, the previous offset value will be replaced by the value being measured at the time.*

5. Change back to the display "Intensity I1". An empty spectrum appears here.

6. Remove the black blocks. The spectrum of the lamp is now visible again.

7. Fill a cuvette with water. This is the reference solution. Place the cuvette into the compact spectrometer.

*Note: Strictly speaking, the reference solution must also contain the buffer substances. However, this is not necessary for measurements of this accuracy.*

8. Change to the display "Reference I2". The spectrum shown here serves as a reference spectrum for the following measurements.

9. Change to the display "Intensity I1". Here the spectrum obtained after the light passes through the solution can be seen. The reference spectrum is also shown in grey.

10. Change to the display "Transmission T". This now shows 100 % at every wavelength. This is the maximum possible amount of light. Any change in the amount of light is now caused only by the dye in the solution.

11. Change to the display "Absorbance E". The absorbance (optical density) is calculated and shown here. All further measurements are made in this display.

## Performing the experiment

### Preparation of the solutions to be measured

1. Place three small drops of bromothymol blue solution into each of the 5 cuvettes.

2. Into each of the cuvettes place 3 mL of the solutions of the various pH values (hydrochloric acid, the buffer solutions with pH values 6.0, 7.0 and 8.0, and sodium hydroxide solution). Ensure that the solutions are mixed well.

*Note: All cuvettes can be pipetted into using the same pipette. Using water prepared ready in a beaker, rinse these once between the measurements. No changes in the pH value will result owing to the effect of the buffer.*

### Recording the pH-dependent spectra of bromothymol blue.

1. Insert the cuvettes one after the other into the compact spectrometer. Start with the solution with the lowest pH value (hydrochloric acid) and end with the solution with the highest pH value (sodium hydroxide solution).

2. The spectrum of the solution appears as soon as the cuvette is inserted. Save this by pressing the record button . Then insert the next cuvette.

### Ending the measurements

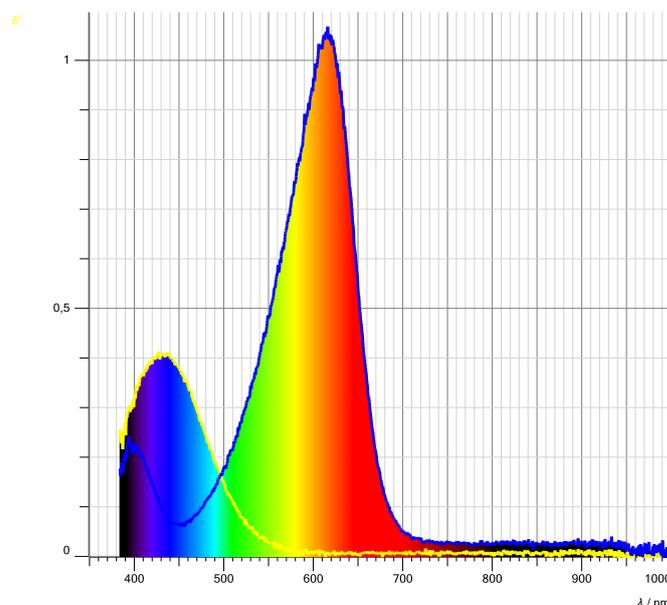
1. End the measurements by pressing "Stop" .

2. Switch off the lamp of the compact spectrometer by clicking on .

## Observation

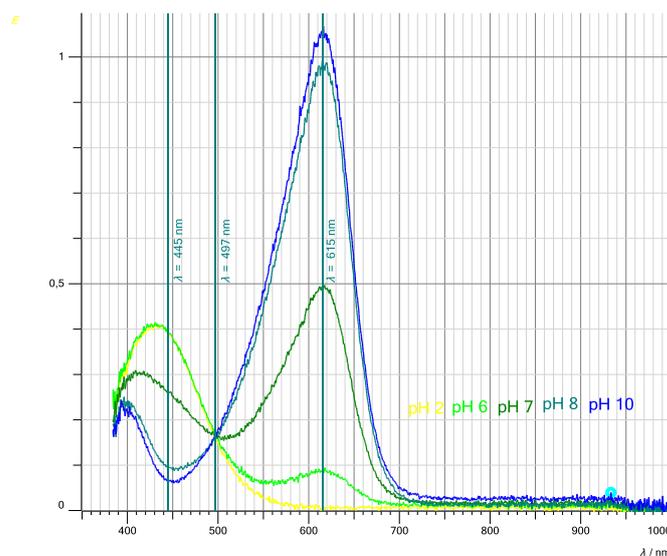
The bromothymol blue solutions have different colours. The hydrochloric acid solution is yellow. Colour sequences from green to blue (sodium hydroxide solution) then follow.

Thus for the hydrochloric acid solution, a spectrum with a maximum at c. 430 nm is displayed in SpectraLab (see Fig. 2). SpectraLab shows which wavelengths have been absorbed by the coloured area under the curve. The yellow solution (hydrochloric acid) absorbs only blue wavelengths, the blue solution (sodium hydroxide solution) absorbs mainly yellow and red wavelengths. Here the maximum is at c. 615 nm.



**Fig. 2:** Spectra of bromothymol blue at pH 2 (yellow) and pH 10 (blue).

The solution with pH 6.0 already shows this second maximum (see Fig. 3). The higher the pH value becomes, the more pronounced is this maximum. The solution with sodium hydroxide solution (c. pH 10) alone produces a maximum at c. 615 nm and has a minimum at c. 445 nm.



**Fig. 3:** Spectra of bromothymol blue at the pH values 2, 6, 7, 8 and 10.

A point at c. 497 nm can clearly be seen where all spectra show the same absorbance.

## Evaluation

### The absorbance maxima

The maximum of the blue solution (in the yellow wavelength range) is at c. 615 nm (see Fig. 3). The larger the amount of blue in a solution, the greater is the maximum. If no blue is contained in the solution, the absorbance at this wavelength will be equal to 0.

The situation is different with the yellow solution. The absorbance maximum here is at  $\lambda = 440$  nm. However, the absorbance minimum of the blue solutions is at  $\lambda = 445$  nm. In order to determine the ratio of the two forms of bromothymol blue in each solution, this absorbance minimum is used.

### Calculating the $pK_a$ value of bromothymol blue

The table with all spectra is copied into a spreadsheet program for further evaluation. To do this, click with the right mouse button on the table and choose "Copy table". Paste the data into the spreadsheet.

Plot the absorbances of the spectra at  $\lambda = 445 \text{ nm}$  and  $\lambda = 615 \text{ nm}$  against the pH value (see Fig. 4). The absorbance at 445 nm corresponds to the concentration of the protonated form HInd, which begins to fall from a pH value of 7. The absorbance at 615 nm corresponds to the non-protonated form Ind, which then begins to rise analogously. At pH 2 the protonated form HInd is present 100 %, the non-protonated form Ind is present 100 % at pH 10. At all other pH values, the ratio lies between these extremes and can be calculated from the absorbance.

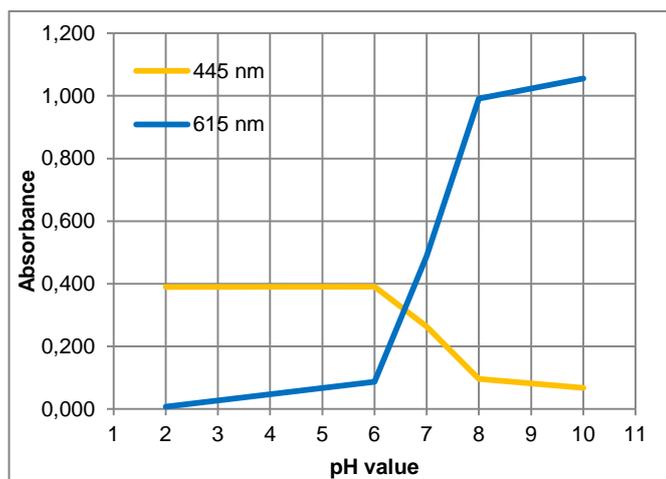


Fig. 4: Plot of the absorbance against the pH value.

For the determination of the relative concentration, the absorbance at the end points (pH 2 and pH 10) is subtracted from all other measured values. The absorbance is linear to the relative concentration of both forms. The  $pK_a$  value can therefore be calculated using the values measured at 445 nm and 615 nm.

In order to calculate the ratio of Ind to HInd, the ratio is calculated between the absorbance at a certain pH value and the maximum absorbance. At  $\lambda = 615 \text{ nm}$  the following applies:

$$\frac{[\text{Ind}^-]}{[\text{HInd}]} = \frac{E - E_{\text{pH}2}}{E_{\text{pH}10} - E}$$

Where  $E$ : is the absorbance (pH-dependent)  
 $E_{\text{pH}2}$ : is the absorbance at pH 2  
 $E_{\text{pH}10}$ : is the absorbance at pH 10

The calculation can be made analogously at  $\lambda = 445 \text{ nm}$ :

$$\frac{[\text{Ind}^-]}{[\text{HInd}]} = \frac{E_{\text{pH}2} - E}{E - E_{\text{pH}10}}$$

The Henderson-Hasselbalch equation is:

$$\text{pH} = \text{p}K_s + \log \frac{[\text{Ind}^-]}{[\text{HInd}]}$$

If the pH and the ratio of  $[\text{Ind}^-]/[\text{HInd}]$  are known, the  $pK_a$  value can be determined from this equation. To do this, plot the pH value against the logarithm of  $[\text{Ind}^-]/[\text{HInd}]$ . A straight line is produced whose intercept corresponds to the  $pK_a$  value of bromothymol blue (see Fig. 5)

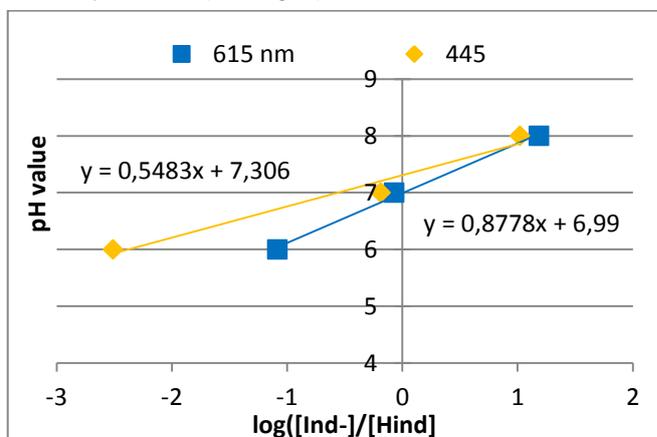


Fig. 5: Plot of the pH value against  $\log([\text{Ind}^-]/[\text{HInd}])$ . Blue values: measured at 615 nm, yellow values: measured at 445 nm.

The  $pK_a$  value is 7.0 when measured at 615 nm and 7.3 when measured at 445 nm.

## Result

### The colour of the solutions

The yellow solution (pH 2) absorbs at blue wavelengths (absorbance maximum c. 430 nm). The blue parts of the white light are absorbed ("filtered out"). As a result, the solution appears in the complementary colour yellow. This applies analogously to the blue solution (pH 10). Here the yellow part of the light is filtered out.

### The isobestic point

All spectra exhibit the identical absorbance at a certain wavelength. This point is referred to as the isobestic point. The absorbance at this point is always constant, independent of the pH value. The isobestic point is proof that in the case of these various coloured solutions, we are dealing with just one dye. This point is around  $\lambda = 497 \text{ nm}$  for bromothymol blue.

### The $pK_a$ value for bromothymol blue

By recording the spectra, it was possible to determine the  $pK_a$  value for the indicator bromothymol blue. It lies between 7.0 and 7.3. The literature value is 7.1 and so agrees with the values measured here.

The measurement becomes more precise if more values are measured in the area of the point of inflection. To achieve this, simply prepare more buffer solutions. The measurement itself hardly takes any longer as a result.

## Cleaning and disposal

The coloured solutions can be disposed of in the laboratory drain with plenty of water. The prepared solutions can be used for further experiments.