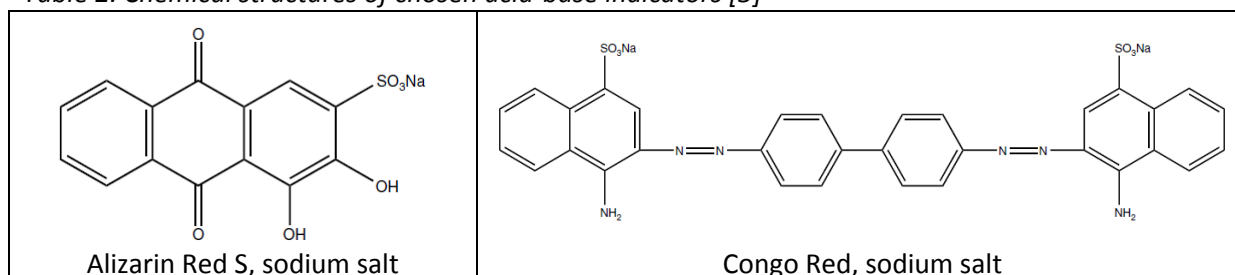# 20. Thin-layer chromatography of acid-base indicators[*]

Acid-base indicators. Acid-base indicators dyes or pH indicators dyes are substances, which are used in chemistry laboratories to visualize end-points of a acid-base titration experiment through color change(s) in different pH environments [1,2]. They constitute a group of organic compounds, like weak acids or weak bases, which react with water and create acid-base conjugate pairs, where both forms are differently colored. In acidic solution protonated structural forms of the dyes exist, whereas in basic solutions deprotonated forms exist. The key structural feature that varies between the acidic and basic forms for acid-base indicator dyes is change in the electronic conjugation (i.e. alternative resonance structures) throughout these molecules. The change in electronic conjugation in various pH environments provide the color changes to 'indicate' a titration end-point. Highly conjugated systems generally absorb and reflect specific wavelengths of light in visible region of the electromagnetic spectrum.

pH indicator dyes can be one-colored (e.g. phenolphthalein), when only one form is colored, second or other forms are colorless, two-colored (e.g. methyl orange) or multi-colored (e.g. alizarin red S), when each form of the indicator dye is differently colored. Sometimes mixtures of indicator dyes are used, which gradually change the colors in the broad range of pH – these kinds of indicators are known as universal indicators. Fluorescent indicators also exist, which change the color of fluorescence or start to fluoresce at specific pH of solution.

Table 1. Colors and pH ranges of color change of chosen acid-base indicators [1,2,3]

Indicator Dye	pH range of color change	Color in solution	
		Acidic solution	Basic solution
Alizarin Red S	3.5-6.5 9.4-12.0	yellow-red	orange-violet
Congo Red	3.0-5.0	blue-red	red
m-Cresol Purple	1.2-2.8 7.4-9.0	red-yellow	yellow-purple
Bromophenol Blue	3.0-4.6	yellow-blue-purple	blue-purple
Rosolic Acid	6.6-8.0	yellow	red
Methyl Orange	3.0-4.4	red-yellow	yellow
Erythrosin B	0.0-3.6	orange-reddish fluorescence	reddish fluorescence
p-Nitrophenol	5.6-7.6	colorless	yellow
Thymol Blue	1.2-2.8 8.0-9.6	red-yellow	yellow-blue
2,6-Dichloroindophenol	no data	red	blue
Methyl Yellow	2.9-4.0	red-yellow	yellow

Table 2. Chemical structures of chosen acid-base indicators [3]

| Alizarin Red S, sodium salt | Congo Red, sodium salt |

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[*] Instruction based on: Daniel D. Clark, Analysis and identification of acid-base indicator dyes by thin-layer chromatography, *Journal of Chemical Education* **84** (2007) 1186-1187.

m-Cresol Purple	Bromophenol Blue
Rosolic Acid	Methyl Orange, sodium salt
Erythrosin B	p-Nitrophenol
Thymol Blue	2,6-Dichloroindophenol, sodium salt
Methyl Yellow	

Thin-layer chromatography. Thin-layer chromatography (TLC) is arguably one of the fastest, simplest, cheapest and most frequently employed analytical techniques, applied to determine the presence and/or purity of the compound in a solution through the separation of the components in a mixture. TLC facilitates the separation of components in a mixture through the unequal distribution (partitioning) of the components in a mixture between a stationary phase and a mobile phase. In normal thin-layer chromatography, the stationary phase is composed of silica gel, aluminum oxide or diatomaceous earth, which are very polar, adsorbed on glass, aluminum or polyester, and the mobile phase (eluent) is virtually any solvent or combination of solvents that are shown to provide the desired separation. During a TLC experiment, a very small amount of a solution is spotted, using a small capillary tube, onto the silica gel (stationary phase) on a TLC plate and this plate is placed in a TLC development chamber containing the eluent (mobile phase). As the eluent rises up the TLC plate, the components in the solution partition between the silica gel and the eluent, on the basis of electrostatic interactions, to afford the separation of the components. The extent of the separation of the components is determined through the calculations of the retention factor value (R_f), a value characteristic to a particular compound. The R_f values are calculated

as a quotient of a distance traveled by a compound and distance traveled by the eluent (solvent front) from the origin (start) line. If the separated compounds are naturally colorless, their presence on the TLC plate can be visualized by lighting with UV lamp or the plate can be sprayed, dipped or developed in the specific solution or vapors or they can be visualized by pyrolisis.

Aim of activity. The aim of this activity is chromatographic separation of the mixture of two acid-base indicator dyes on the TLC plates and their identification.

EXPERIMENTAL SECTION

Caution. During analysis flammable organic solvents and concentrated ammonium hydroxide are used. Safety precautions should be taken. Avoid sources of an open fire. Solvents must be disposed of in appropriate waste containers. Preparation of the eluent solution and TLC plates development must be performed in the vented hood.

Advices for optimal TLC experiment.

➢ Be gentle with TLC plates and do not directly touch the silica surface with your hands – you can contaminate the surface – always hold the TLC plates by their sides or with pincette.

➢ Start (origin) line, front (eluent) line and the separated spots mark only with pencil – never use ballpen, pen or marker because the ink can contaminate the surface during plate developing.

➢ Use pencil and capillary very gently – do not destroy the silica layer on the plate.

➢ Spot a very small amount of sample with capillary on the TLC plate – spots shouldn't have more than 2 mm in diameter – look at the ruler!

➢ Each time spot the sample with a new capillary to avoid contamination of the reagents and TLC experiment.

➢ Allow the sample solvent to dry for several minutes prior to development of the TLC plates – residual sample solvent can dramatically influence chromatographic properties.

Materials	Chemicals
- flask with ground glass joint per 100 ml,	- propan-1-ol, $CH_3CH_2CH_2OH$,
- graduated cylinders per 25 ml,	- 28 % ammonium hydroxide solution, $NH_{3(aq)}$,
- graduated cylinders per 10 ml,	- 1 M hydrochloric acid solution, HCl - sprayer
- 2 beakers per 250 ml,	- methanolic solutions of each individual pH indicator,
- 2 Petri dishes,	- methanolic solution of all pH indicators.
- 3 TLC plates,	
- capillaries,	
- plastic pincette,	
- metric ruler,	
- pencil,	
- hair dryer.	

PROCEDURE

Preparation of the eluent solution. In order to prepare eluent solution mix together propan-1-ol and 28% ammonium hydroxide solution in 6:1 volume ratio to obtain about 50 ml of solution. If the experiment takes more then 1,5 hour prepare new and fresh sample of eluent.

Analysis. Draw a straight origin line across each of three obtained TLC plates, in pencil, about 1 cm from the bottom of each plate – see Figure 1.

Using a small capillary spot a very small sample (about 2 mm in diameter) of your analyzed solution on the origin line on one plate. 1 cm away from the first spot, spot a very small sample (about 2 mm in diameter) of the solution of the **mixture of all dyes** on the origin line of the plate (use a new capillary). Allow the spots to dry for about 5 minutes. Pour about 20 ml of the eluent solution to the development chamber (a beaker). Place carefully the TLC plate in the chamber, cover the beaker with Petri dish and develop the plate until the eluent has traveled 4/5 of the length of the plate. Pull out the TLC plate from the chamber, mark the solvent front and all of the separated spots with a pencil. Draw a clearly labeled sketch of the developed TLC chromatogram in your lab-book – mark origin line, solvent front, spots positions, their colors (note the

colors while the TLC plate is still wet) and other interesting features. Calculate the R_f values for each spot corresponding to the separated components. Compare the observed colors and calculated R_f values for each spot corresponding to the separated components of the analyzed sample to the known colors of the compounds under basic/high pH conditions (the TLC eluent has a pH around 9-10) and R_f values provided in Table 3 to make a preliminary hypothesis of the identities of the components in the analyzed mixture.

Table 3. TLC properties of acid-base indicator dyes. R_f values are for stationary phase Silufol® Avalier Czechoslovakia ON684080 JK253422

Indicator dye	R_f	Spot color on the developed TLC plate	
		Basic solution	Acidic solution
Alizarin Red S	0.00	violet	light yellow
Congo Red	0.30	orange	purple
m-Cresol Purple	0.30	gray-purple	red
Bromophenol Blue	0.40	dark blue	yellow
Rosolic Acid	0.50	pink	yellow
Methyl Orange	0.60	yellow	red
Erythrosin B	0.70	hot pink	light orange
p-Nitrophenol	0.80	light yellow	colorless
Thymol Blue	0.75	mustard	purple
2,6-Dichloroindophenol	0.85	indigo	light violet
Methyl Yellow	0.90	yellow	red

Second and third TLC plates should be used to determine the accuracy of the initial hypothesized identity of the analyzed sample mixture. Using a small capillary spot a very small sample (about 2 mm in diameter) of your analyzed solution on the origin line of the second plate. 0.5 cm away from the first spot, spot a very small sample (about 2 mm in diameter) of the solution containing pure, known dye X which is hypothesized by you to be present in your analyzed mixture solution on the origin line of the plate (each time use a new capillary). Next 0.5 cm away from the second spot (third spot), co-spot the analyzed mixture and pure, known dye X. Similarly spot on the origin line of this plate second solution containing pure, known dye Y which is hypothesized by you to be present in your analyzed mixture solution (fourth spot) and next co-spot your analyzed sample and the second known dye Y (fifth spot) – see Figure 1. Repeat the whole procedure again on the third TLC plate.
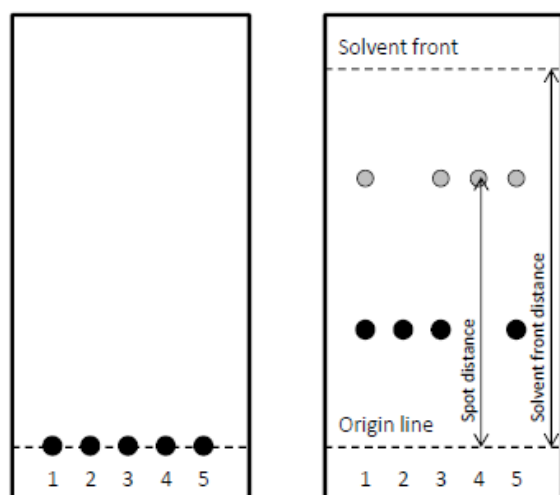

Figure 1. Schematic depiction of the TLC plate prepared for the TLC experiment and the TLC experimental results confirming the correct identification of the components in the mixture. Spot 1: Analyzed mixture. Spot 2: Solution containing pure, known dye X which is hypothesized to be present in the analyzed mixture. Spot 3: Co-spot of the analyzed mixture and dye X. Spot 4: Solution containing pure, known dye Y which is hypothesized to be present in the analyzed mixture. Spot 5: Co-spot of the analyzed mixture and dye Y.

Allow the spots to dry for about 5 minutes. Pour about 20 ml of the eluent solution to the second and third development chambers (beakers). Place carefully the TLC plates in the chambers, cover the beakers with Petri dishes and develop the plates until the eluent has traveled 4/5 of the length of the plates. Pull out the TLC plates from the chambers, mark the solvent fronts and all of the separated spots with a pencil. Draw a clearly labeled sketch of the developed TLC chromatograms in your lab-book – mark origin lines, solvent fronts, spots positions, their colors (note the colors while the TLC plates are still wet) and other interesting

features. Calculate the R_f values for each spot corresponding to the separated components. Dry the plates. The results from this experiment can provide support for your identification of one or both of the components in the analyzed sample on the basis of appearance and co-migration (same apparent R_f values) on the TLC plates. If neither or only one of the compounds were identified, repeat the experiment with different, known dyes solutions until the identity of both of the components in the analyzed sample are confirmed and supported by TLC data.

Additional evidence to support the identity of the components in the analyzed sample can be performed by gentle spraying of the dry plate number 3 by acidic 1 M hydrochloric acid solution (pH<1). Note in your lab-book the color changes of your spots. Compare now these colors with the colors of the hypothesized dyes in acidic environment provided in Table 3.

Calculations. The retardation factor R_f for each substance can be calculated from the following formulae:

$$R_f = \frac{a}{b}$$

where a is the distance from start (origin) line to the spot center, b is the distance from start (origin) line to the front of solvent (eluent).

Report

The report should be composed of:

- ➢ first name and last name of the person conducting the analysis,
- ➢ analysis' date,
- ➢ clearly labeled drawings of each developed chromatogram,
- ➢ analysis results – number of identified indicator dyes in received sample together with the details supporting these identifications – i.e. calculated R_f values, colors, color changes under the effect of HCl and etc.,
- ➢ possible comments.

Sources:

1. Jerzy Minczewski, Zygmunt Marczenko, Chemia analityczna 1. Podstawy teoretyczna i analiza jakościowa, Wydawnictwo Naukowe PWN, Warszawa 2004; Jerzy Minczewski, Zygmunt Marczenko, Chemia analityczna 2. Chemiczne metody analizy ilościowej, Wydawnictwo Naukowe PWN, Warszawa 2004
2. Ram W. Sabnis, Handbook of acid-base indicators, CRC Press, Taylor and Francis Group, 2008
3. http://www.sigmaaldrich.com