Name of the project:	**Digitization of chemistry experiments to improve the quality and support chemistry teaching in secondary schools**
Acronym:	**ChemIQSoc**
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Tittle: SDS-PAGE electrophoretic separation of proteins

Work instructions

Task: Separate the fractions of storage proteins in SDS-PAGE and evaluate them using standards (marker).

Theory

Electrophoretic methods are physical-chemical methods that are used to separate substances carrying electric charges. When a mixture of such substances is exposed to an electric field in a certain environment, the molecules move. Their mobility depends on the size of the charge, the size and shape of the molecules, the conditions of the environment (for example, the character of the carrier) and the strength of the electric field. The size of the molecule's charge is influenced by the degree of ionization, pH and ionic strength of the environment.

Electrophoresis can be used to separate low-molecular and high-molecular substances. In molecular biology, agarose and polyacrylamide gels are most often used as carriers for separating substances. Gels have the character of molecular nets, which allows to also separate such substances that have the same charge, but different size of molecules. According to the appropriate gel, we distinguish agarose and polyacrylamide electrophoresis (PAGE). Polyacrylamide electrophoresis (PAGE) can be used to separate nucleic acids and proteins. Polyacrylamide gel has very good mechanical properties, it is transparent, during preparation it is possible to ensure the required pore size, gel structure is very well reproducible, it has the highest resolution capacity of all carriers. Polyacrylamide gel is formed by polymerization of basic acrylamide monomer and cross-linking monomer N,N'-methylene-bis-acrylamide. Acrylamide and BIS are toxic, so they must be handled carefully! The polymer is no longer toxic.

The basic device for electrophoresis consists of an electrophoretic chamber and a source of direct electric current. The electrophoretic chamber contains electrolyte and space for placing gel. Polyacrylamide gel is prepared in the form of plates on glasses with different dimensions, which are in vertical position during electrophoresis.

For separation of proteins according to molecular size, PAGE is used in sodium dodecyl sulfate (SDS) environment. SDS binds to peptide bonds and basic groups of proteins, as a result all proteins acquire almost equally large negative charge (during electrophoresis they move to anode) and during electrophoresis they are then separated only according to size of their molecules–smaller molecules move faster, larger molecules slower. If SDS and reducing agents (2-mercaptoethanol, dithiothreitol) are added to proteins with subsequent thermal denaturation,

their three-dimensional conformation is disrupted, and molecules assume approximately same shape. Proteins can also be separated without SDS presence, in this case proteins are not separated according to molecular size (so-called native PAGE). During protein electrophoresis using SDS-PAGE, a gel consisting of two concentration inhomogeneous parts is prepared–a more concentrated separating gel (in lower part of plate) and a less concentrated starting gel. Gel staining is done with specific dyes, e.g. Coomassie Brilliant Blue R-250 or staining with silver nitrate (silver staining).

Equipment: test tubes, automatic pipettes, tips for automatic pipettes, apparatus for vertical discontinuous electrophoresis Biometra, source of direct electric current

Chemicals: TRIS base, acrylamide, bisacrylamide, sodium dodecyl sulfate (SDS), ammonium peroxodisulfate (APS), N,N,N',N'-tetramethylenediamine (TEMED), trichloroacetic acid, Coomassie Brilliant Blue R-250, ethanol

Solutions

1M Tris-HCl pH 6.8	12.114 g Tris base make up to 100 cm^3 with water and pH adjusted with HCl
1M Tris-HCl pH 8.8	12.114 g Tris base make up to 100 cm^3 with water and pH adjusted with HCl
A-BIS starting	7.29 g Acrylamide + 0.125 g Bisacrylamide make up to 100 ml with d. water AA-BIS
AA-BIS separating	54.49 g Acrylamide + 0.72 g Bisacrylamide make up to 250 ml with d. water 10% SDS
10% SDS	5 g SDS make up to 50 ml with distilled water
2% APS	0.02 g PSA + 1 ml of redistilled water (fresh every day!)
Electrode solution	28.2 glycine + 6 g Tris + 2 g SDS make up to 2 l with distilled water

Composition of 100 ml separating gel

38.1 ml	of 1 mol/dm^3 Tris-HCl, pH 8.8
58.27 ml	of solution of BIS-acrylamide (AA-bis), (12.7g acrylamide + 0.168 g N,N'-methylenebisacrylamide in volume of 58.27 ml)
1 ml	of 10% solution of SDS
2.53ml	of 1% solution of ammonium persulfate

50 µl TEMED	After polymerization of the separating gel, remove butanol and pour the starting gel up to the upper edges of the glass plates.

Composition of 20 ml starting gel

2.47 ml	of 1 mol/dm^3 Tris-HCl, pH 6.8
16.6 ml	of solution of AA-BIS (1.21 g acrylamide + 20.8 mg N,N'-methylenebisacrylamide in volume of 16.6 ml)
0.2 ml	of 10% solution of SDS
741 µl	of 1% solution of ammonium persulfate
20 µl	of TEMED

Composition of electrophoretic solution

- 3 g of Tris-HCl
- 14.1 g of glycine
- 1 g of SDS 10%, dissolve and make up to 1000 ml with distilled water, adjust pH = 8.3

Composition of staining solution

- 95 ml of 10% trichloroacetic acid and 5 ml of 0.5% solution of Coomassie Brilliant Blue R-250 in ethanol

Procedures:

Prepare the apparatus for electrophoresis

1. Clean the glass plates with warm water and ethanol.
2. For the preparation of plates, we use two unevenly sized glasses.
3. After thorough cleaning, we join them with clamps.

Gel preparation

1. Prepare gels in glass plates: separating and focusing.
2. We pour the separating gel about 1.5 cm below the upper edge of the smaller plate. To level the surface of the gel, we drip 3-5 drops of butanol. We let the separating gel polymerize for 30 minutes.
3. After polymerization of the separating gel, we remove butanol and pour the starting gel up to the upper edge of the glass plates.
4. The starting gel polymerizes very quickly (within 1 minute), so it must be poured into the plates as soon as possible after preparation. After the solidification of the starting

gel, we remove the combs from the plates. Into the wells that remained in the starting gel after the comb, we apply samples in an amount of 5 µl. We place the prepared plates with applied samples in the electrophoretic chamber and add the electrode solution.

Protein Separation

1. Electrophoretic separation takes place at a current size of 30 mA for 6 to 8 hours, at a constant temperature of 15°C, until the marker reaches the bottom edge of the gel.
2. The first 15 minutes the separation takes place at a current size of 5 mA, another 25 minutes at 10 mA and other hours at a current size of 40-60 mA.

Staining of gel and visualization of proteins

1. All protein fractions separated in SDS-PAGE are stained in a solution prepared by mixing 95 ml of 10% trichloroacetic acid and 5 ml of 0.5% solution of Coomassie Brilliant Blue R-250 in ethanol. Excess dye from the gel is removed by washing gels in distilled water for 16 hours.

Management of chemical substances

Chemicals	**Form**	**H-statements**	**P-statements**
HCl	Liquid	H314, H335	P261, P280, P305, P351, P338, P304, P340, P310
C_2H_5OH	Liquid	H225	P210, P233
Acrylamide	Solid	H301, H317, H340, H350, H361f, H372	P201, P280, P302, P352, P308, P313
Bisacrylamide	Solid	H301, H340, H350, H361fd, H372	P260, P280, P301, P31, P308, P313
Sodium dodecyl sulfate (SDS)	Solid	H228, H311, H319, H335	P210, P261, P305, P351, P338, P280, P312
Ammonium peroxodisulfate (APS)	Solid	H272, H302, H315, H317, H319, H302, H334, H335	P261, P280, P302, P313, P332, P338, P351, P352
N,N,N',N'-tetramethylethylene diamine (TEMED)	Liquid	H225, H302, H314, H331	P210, P280, P312, P303, P361, P353, P304, P340, P310, P305, P351, P338

Chemicals	Form	H-statements	P-statements
Coomassie Brilliant Blue	Solid	H319, H335	P261, P280, P305, P351, P338, P403, P233, P405, P501
Trichloroacetic acid	Liquid	H314, H335, H400, H410	P260, P280, P303, P304, P361, P353, P340, P310, P305, P351, P338

Sources of risk and assessment of risk severity

Most of the chemicals used to prepare the gel are hazardous and classified as poisons; therefore, we work with them very carefully and using all the rules for working with hazardous substances. We use personal protective equipment (gloves, goggles, lab coat).

Waste management method

Waste generated during the analysis is disposed of in containers designated for this purpose.

Risk reduction measures

Use of personal protective equipment (goggles, gloves, coat). For safety and health protection at work, it is advantageous to buy ready-made polyacrylamide gels, which are no longer toxic.

References

1. CHŇAPEK, M.: *Využitie bielkovinových markerov pri identifikácii, diferenciácii a charakteristike genotypov pšenice letnej, tvrdej, špaldy a jačmeňa jarného*. Doktorandská dizertačná práca. Nitra: SPU, 2007.

Worksheet

Experimental data

1. Record current values at 15 min time interval

Time [min]	Current [mA]	Time [min]	Current [mA]
0:15		4:15	
0:30		4:30	
0:45		4:45	
1:00		5:00	
1:15		5:15	
1:30		5:30	
1:45		5:45	
2:00		6:00	
2:15		6:15	
2:30		6:30	
2:45		6:45	
3:00		7:00	
3:15		7:15	
3:30		7:30	
3:45		7:45	
4:00		8:00	

2. Measure the distance of the electrophoretic cell (gel height) from the beginning to the end *l*

Calculations

1. Record the spot distances of the standards (from the beginning to the centre of the spot) for each protein standard *a*

Molecular mass	*a* [mm]	Molecular mass	*a* [mm]

2. Record and compare the spot distances of the proteins in the samples with the standards and identify the individual proteins.

Questions

1. According to what separation principle are proteins separated in SDS-PAGE electrophoresis.

2. What is the staining solution used for?

3. Describe what working electrophoretic parameters affect the distance travelled by proteins in an acrylate gel.

4. Write what parts/modules the electrophoretic device consists of.

5. Describe the individual electrophoretic principles.

6. Indicate the errors and their sources in electrophoretic determination of proteins. Suggest possible solutions.

Conclusion

Briefly summarize the objective of the experiment, the main results and compare them with the expected values.

Disclaimer

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