**INSTRUMENTAL ANALYSIS LABORATORY**

**NAME OF THE EXPERIMENT:** TLC-PC-ELECTROPHORESIS

**DATE OF THE EXPERIMENT:** 8.05.2023 – 9.05.2023

**NAME OF THE ASSISTANT:** Buse Ayşen Dündar

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**GROUP NUMBER:** 6

**DATE SUBMISSION:** 17.05.2023

**INSTRUMENTATION**

**TLC**

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End Point

Reference Point

**Tank Silica Plate**

Lid is used to prevent the evaporation, of the mobile phase. It is used, after adding a silica, plate to the mobile phase.

Solvent Tank: plate and mobile are placed in.

Solvent: It is located at the bottom of the tank as a mobile, phase solvent. It separates molecules or compounds found in the designated places on the plate (indicated by a dot).

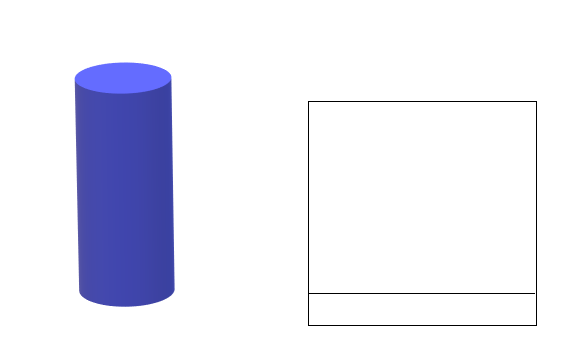
Silica plate: Rf value is calculated by maesuring the distance the compounds placed on the, reference points on this plate move. ,Its structure is prepared by spreeding the glass layer on the metal.

**PC**

It is the place where the stationary phase paper is placed in the cylindrical tank. Solvent is also found here.

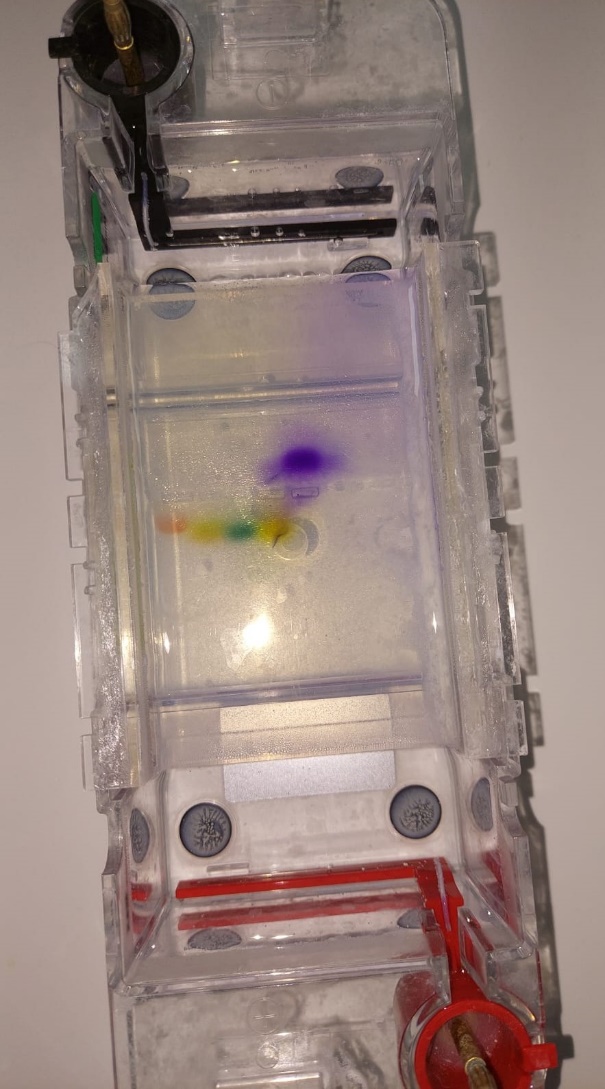
In paper chromatography, the stationary phase is cellulose paper. The locations of the compounds are marked on the reference line. The paper is rolled up, to fit the cylinder tank and placed inside the tank. ,The paper should be placed in such a way that it does not tuoch the sides of the cylinder, tank as much as possible.

The solvent, that is, the mobile phase, should move smoothly on the paper and reach the, highest point it can reach**.**

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**Cylindrical Tank Paper**

**ELETROPHORESIS**

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The casting tray is placed over the electrophoresis chamber.

The buffer solution should be placed so that it passes over the agarose gel to achieve conductivity.

Other tasks of the buffer solution include increasing the ionic strength and keeping the pH constant.

Comb serves to monitor separation.

The power source creates a power difference between the electrodes, allowing the charges to move.

**CALCULATIONS**

**TLC**

**Picture 1.** TLC Chromatography 1st mobile phase,

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**Rf =**

The distance of solvent front from origin which is 3 cm and same for all dyes,

For C which is represents the Congo red

Rf == 0,43

Calculating the Rf values for all dyes the same calculation process then

**Table1**.Dyes, the distance on the plate,Rf  values

|  |  |  |  |
| --- | --- | --- | --- |
| **Dyes** | **Compound distance from origin (cm)** | **distance of solvent from origin (cm)** | **Rf** |
| **C: Congo Red** | **1,3** | **3** | **0,43** |
| **P: Phenol Red** | **3** | **3** | **0,7** |
| **B: Bromophenol Blue** | **2,1** | **3** | **0,98** |
| **CV: Cresyl Violet** | **2,9** | **3** | **0,53** |
| **BCB: Brilliant Cresyl Blue** | **1,6** | **3** | **0,33** |
| **Unknown (1)** | **2,9** | **3** | **0,98** |
| **Unknown (2)** | **2** | **3** | **0,67** |
| **Unknown (3)** | **1,5** | **3** | **0,5** |

**Picture 2.** TLC Chromatography, 2nd mobile phase,

**metin, ölçüm çubuğu, cetvel, iç mekan içeren bir resim

Açıklama otomatik olarak oluşturuldu**

**Rf =**

The distance of solvent front from origin which is 3,1 cm and same for all dyes,

For C which is represents the Congo red

Rf == 0,52

Calculating the Rf values for all dyes the same calculation process then

**Table 2.** Dyes, the distance on the plate,Rf  values

|  |  |  |  |
| --- | --- | --- | --- |
| **dyes** | **Compound distance from origin (cm)** | **distance of solvent from origin (cm)** | **Rf** |
| **C: Congo Red** | **1,6** | **3,1** | **0,52** |
| **P: Phenol Red** | **2,3** | **3,1** | **0,74** |
| **B: Bromophenol Blue** | **3** | **3,1** | **0,97** |
| **CV: Cresyl Violet** | **1,4** | **3,1** | **0,45** |
| **BCB: Brilliant Cresyl Blue** | **0,9** | **3,1** | **0,29** |
| **Unknown (1)** | **3** | **3,1** | **0,97** |
| **Unknown (2)** | **2,3** | **3,1** | **0,74** |
| **Unknown (3)** | **1,3** | **3,1** | **0,42** |

In Rf values which is come from TLC based on the comparison of reference values, it is seen, that the value found for Unknown(1) has the same Rf values as Bromophenol Blue.It is seen that the Rf ,value of Unknown(2) is the same, as the Rf value of phenol red. It is seen that the Rf value for Unknown (3) is close to the Rf value of Cresyl Violet. As a result of these comperisons, it is concluded that the mixture contains Cresyl Violet, Phenol Red, and Bromophenol Blue.

**PC**

**Picture 3.Paper Chromatography of Ni2+ , Cu2+ , Co2+,**

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**Rf =**

The distance of solvent front from origin which is 10 cm and,same for all metal ions

For Ni2+

Rf == 0,3

Calculating the Rf values for all dyes the same calculation process then

**Table 3.** Metal ions, the distance on the paper,Rf  values

|  |  |  |  |
| --- | --- | --- | --- |
| **Metal Ions** | **Compund distance from origin (cm)** | **distance of solven from origin (cm)** | **Rf** |
| **Ni** | **3,3** | **10** | **0,3** |
| **Cu** | **5,4** | **10** | **0,54** |
| **Co** | **3,9** | **10** | **0,42** |
| **Unknown (1)** | **5,5** | **10** | **0,55** |
| **Unknown (2)** | **2,9** | **10** | **0,29** |

In PC, based on the Rf values, Rf for Unknown(1) is calculated as 0.55, which is very close to ,the Rf experience of Cu metal.The Rf value for unknown (2) is 0.29, which is pretty close to, the Rf life of Ni metal.

**ELECTROPHORESIS**

**Picture 4. Electrophoresis of Ni2+ , Cu2+ , Co2+**

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generated](data:image/jpeg;base64,/9j/4AAQSkZJRgABAQEAYABgAAD/4S5uRXhpZgAATU0AKgAAAAgABgALAAIAAAAmAAAIYgESAAMAAAABAAEAAAExAAIAAAAmAAAIiAEyAAIAAAAUAAAIrodpAAQAAAABAAAIwuocAAcAAAgMAAAAVgAAEUYc6gAAAAgAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAFdpbmRvd3MgUGhvdG8gRWRpdG9yIDEwLjAuMTAwMTEuMTYzODQAV2luZG93cyBQaG90byBFZGl0b3IgMTAuMC4xMDAxMS4xNjM4NAAyMDIzOjA1OjE3IDExOjI4OjI1AAAGkAMAAgAAABQAABEckAQAAgAAABQAABEwkpEAAgAAAAM5NAAAkpIAAgAAAAM5NAAAoAEAAwAAAAEAAQAA6hwABwAACAwAAAkQAAAAABzqAAAACAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA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**POST LAB QUESTIONS**

**TLC**

1)The mobile phase, (solvent) compounds used in the TLC seperation method generally have volatile properties. The open side of the solvent tank is closed with a lid to prevent the, evaporation of the mobile phase. At the same time, this process contributes to forming a more stable and consistent mobile step that cannot evaporate immediately. This is why it is essential to prevent wrong evaluations. Closıng the lid prevents evaporation and pollutiion in the air from entering the solvent tank. In the TLC method, there must be no pollution, and even the most minor changes in the plate, can affect the results of the method. Finally ,some mobile phases can be harmful ıf inhaled due to the chemical molecules they contain., Closing the lid of the solvent tank contributes to reducing the damage of the mobile degrees, which are harmful to inhale.

2)To decide which mobile phase is more suitable for dyes separation, it is necessary to consider the Rf values, of dyes in two different mobile stages. In comparison, the Rf value for bromophenol blue, cresyl violet, and bright cresyl has a greater value in the first mobile phase; in this case, the first mobile phase is for these colors. It has better solubility and seperation power because its rf and solubility values of the compounds used in TLC are considered. Congo, Red and Phenol Red, in have higher Rf values in the second mobile phase with Rf values. In line with the reasons explained above, it is seen that the first mobile phase is more suitable for these dyes.

3)The color of bromuphenol blue, can change depending on the pH. Therefore, it is observed that in the pH=4.6 range, blue is yellow at values close to pH=3. The pH value of the second, mobile phase is approximately three. ,Therefore, the color of bromophenol blue in this mobile phase is yellow. Has been observed.

4)The silica plate used for TLC has polar properties. The calculated Rf value gives information about the polarity values for the dyes. ,When the Rf values are compared, the non-polar dye will have an enormuos Rf value since the silica plate has polar propeirties. The dye will be the most, polar because the silica plate is opposing, so the plate will hold it, and its progress on the plate will not be as much as in non-polar dyes. Since the dye stationary phase with the highest Rf value is silica polar, it will be the most nonpolar dye. The dye with the smallest Rf value will be the most polar dye.In this case, the polarity for the dyes will start from the most polar to the most nonpolar, respectively.

Brillıant Creysl Blue > Creysl Vıolet > Congo Red >Phenol Red >Bromophenol Blue

**PC**

**1)** Rubeanic acid contributes to the detection and visibility of metal ions in chromatography, methods. In Paper chromatography, when sprayed on paper, it reacts with the metals on the form and causes color changes to be observed, and it is said to be one of the main reasons for using Rubeanic acid because it craetes color change. Because with this colour change, the separation, of metal ions becomes much more observable and contributes to the identification of metal ions.

**ELECTROPHORESIS**

**1)**

**I) Congo Red**



Congo red compound is a negatively charged compound. Therefore, congo red will move ,towards the positive electrode. The data obtained as a result of the experiment shows this**.**

**II) Phenol Red**



**II) Phenol Red**

The compound is negatively charged because of the , oxygen which is in the SO3, .Therefore, this compound will move towards the electrode with a positive charge. ,This has been proven because of the experiment.

**III) Bromophenol Blue**



Bromophenol Blue, has electronegativity due to OH, Br, S, and O in its structure. ,Due to this feature, it will move towards the positive electrode and this has been proven as a result of the, experiment.

**IV) Methyl Red**



Methyl should move towards, the positive electrode because of the N and COOH it contains in, its red structure.

**V) Cresyl violet**



NH2+, in the Creysl structure adds a positive feature to the system, which indicates that it moves, toward the negative electrode. Although, there is no apparent progress in the painting belonging to the experiment, it can be thougth that the paint color goes in the negative direction.

**VI) Brilliant Cresyl Blue**



Brilliant, cresyl blue is a compound that can be evaluated positively with the O+, it contains in the structure of the mix, and it moves towards the negative electrode. Although it cannot be,, clearly distinguished in the data obtained from the experiment, the same situation is also seen, in cresyl, violet because these colors were not encountered where the positively charged electrode is.,

2) In the electrophoresis method, while the dyes are progressing, the dyes with a large size will, travel a short distance, as it will be difficult to advance in the gel.,Since it will not be diffıcult for them to progress in small-sized dyes gel, they can go over long distances in the gel. No calculation has been made for the spaces observed in the question. Therefore, theoretically, an idea can be made based on ionic strength.

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