Chromatography separation of dye mixture

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Chromatography is a technique used to separate the components of a mixture 1. There are two phases in chromatography, the stationary phase (absorbed solvent) and the mobile phase (moving solvent). The process of chromatography involves passing a mixture dissolved in a “ mobile phase” through a stationary phase. Since each phase has a different distribution coefficient, the components travel at a different rate and thus get separated. The two commonly used techniques of chromatography are thin layer chromatography, TLC, and column chromatography. Thin layer chromatography is used to determine the purity of a substance, to identify, and is used to determine solvent system for separations of mixtures. This technique is especially useful in determining optimum conditions for separating compounds by column chromatography 1. On the other hand, column chromatography is used on a much larger scale. It is used to separate mixtures made of two or more compounds. During column chromatography, these components are separated many times between the stationary phase and the mobile phase.

The purpose of conducting this experiment was to determine the suitable solvents for the various components in a mixture of 1: 1 methylene blue and fluorescein dye. The two eluting solvents used in the experiment were 1: 12: 14 mixture of K2SO4: CH3CN and 95% ethanol. The experiment allowed us to identify the effects of the two different solvents on the different dye mixtures. The effect can be observed from the retention factor, Rf, which is a ratio of the distance traveled by the sample to the distance traveled by the solvent 2. Different conclusions can be drawn up from the Rf value, a high Rf

value would indicate that the substance is less polar and has traveled a greater distance and a low Rf value would indicate the opposite.

The two dye mixtures used in the experiment are methylene blue and fluorescein. Based on the properties of the two substances, the alternate hypothesis that methylene blue will have a higher retention factor compared to fluorescein can be stipulated. It can also be hypothesized that since fluorescein is more polar than methylene blue it will dissolve in the more polar solvent and travel a greater distance.

Results

The distance traveled by each dye mixture, the R- value, is shown in Table 1 and Table 2. These R-values are used to calculate the Rf values for each mixture, which are also shown in Table 1 and Table 2. The Rf values for the mixtures in 1: 12: 14 K2SO4: H2O: CH3CN are 0. 76, 0. 70 and 0. 75 for fluorescein, dye mixture and methylene blue respectively. The Rf values for the mixtures in 95% ethanol are 0. 057, 0. 32 and 0. 34 for fluorescein, dye mixture and methylene blue respectively. As indicated in the tables above, both the eluting solvents, 1: 12: 14 K2SO4: H2O: CH3CN and 95% ethanol separated impurity on the TLC plate. The Rf values are similar with the Rf values found by other experiments. M. B Naff and A. S Naff found the Rf values of fluorescein to be 0. 85 and the Rf value of methylene blue to be 0. 02, when the eluting solvent used is a ratio of 2: 2: 1 methyl ethyl ketone: acetic acid: isopropyl alcohol 1. Table 3 shows the elution of the fluorescein and methylene blue, with methylene blue eluting first followed by fluorescein.

Discussion

Thin layer chromatography was used to determine the most suitable solvent system for the separations of the mixtures. From the data gathered, it was observed that both fluorescein and methylene blue traveled a further distance on the chromatogram when the solvent 1: 12: 14 K2SO4: H2O: CH3CN was used, as compared to the distance traveled when the solvent being used was 95% ethanol. This shows that the solvent 1: 12: 14 K2SO4: H2O: CH3CN is more polar than 95% ethanol since in the solvent 1: 12: 14 K2SO4: H2O: CH3CN both the polar dye mixtures dissolve (like attracts/ dissolves like) and travel a further distance. The polarity of the elutent forces the compounds to the top of the place, because the compounds dissolve well and do not interact with the stationary phase.

In TLC, the adsorbent (stationary phase) is thinly spread onto a flat sheet of supportive plastic.

The mixture to be separated is applied onto the stationary phase about 1 cm from the bottom of the chromatographic sheet. The sheet is then placed into a developing chamber containing the mobile phase. The mobile phase rises up the chromatography sheet by capillary action. As the mobile phase proceeds up the sheet, the components of the mixture are retained in various degrees by the stationary phase. The chemical composition of the stationary phase and the mobile phase play a significant role in how far the components travel up the chromatographic sheet.

In column chromatography, a glass column is packed with a solid stationary phase. The mixture to be separated is applied at the top of the column. The

mobile phase descends by gravity through the column. The components of the mixture to be separated have different properties.

The rate at which the components descend through the column depends on several factors. The component is retained by the stationary phase to a certain extent depending on the properties of the stationary phase and the properties of the component. The solvation power of the solvent also affects the rate of elution. The rule of “ like dissolves like” applies here. The individual components, with different affinities for the stationary phase and the mobile phase, are continuously absorbed onto the stationary phase, solvated by the mobile phase eluting through the column, reabsorbed onto the stationary phase, etc. The speed at which the components travel through the column is directly related to the number of absorption-elution cycles that occur.

Therefore a balance between the solvation power of the mobile phase and the absorption power of the stationary phase determines how fast each individual component travels through the column. 1, 2

Think of a piece of wood floating down a creek. If there is a lot of grass growing in the stream, the wood will get caught in the grass for awhile, then it will break loose and flow down the creek a short distance, get caught in some more grass or rocks, break free again, and continue this process until it has made its way down the creek. Aluminum cans will travel down the creek at different rates than the wood based on the amount of time they are retained by the grass. If there is no grass in the creek, the piece of wood and aluminum can will both reach the end of the creek WRONG!!! From the data

gathered it can also be observed that the polar dye mixture, fluorescein ascended quickly when the solvent 1: 12: 14 K_2SO_4: H_2O: CH_3CN was used. This is primarily because the nonpolar compounds stick to the stationary phase, while polar compounds separate and travel upwards with the solvent. From the TLC plates, it is indicated that different compounds in the mixture travel at different rates; polar compounds travel quickly while lesspolar compounds travel more slowly.

The stationary phase was the substrate alumina which is considered to be a polar substance since the surface consists of polar (OH) groups. The ‘ moving’ phase is the solvent system that, moves up the stationary alumina coated plate. All solvent systems will be considered non-polar relative to the silica adsorbent.

Potential problems leading to yield loss- In between two sand layers some impurities were trapped and on top of alumina fluroscien dye stayed. The 95% ethanol and mixture of blue dye dripped through columns down the container and collection of this clear mixture ended when solvent was colourless. Then sodium hydroxide was used to wash out the fluorescien dye into a separate beaker which caused the purple impurities to move down the cotton. This might be due to the thin layers of sand used or excessive solvent. However, this can be prevented by lowering the time it may take the dye to come down the column by increasing the air pressure from above (Still et al., 1978).

Potential improvements to the process or problems with the experiments

However, this can be prevented by lowering the time it may take the dye to come down the column by increasing the air pressure from above 3.

The two dyes methylene blue and sodium hydroxide were used to separate fluorescien dye with ethanol in second part, column chromatography.

References

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Questions

What would happen if the level of the solvent in your TLC chamber was higher than the spots at the bottom of your TLC plate?

If the level of the solvent in the TLC chamber was higher than the spots at the bottom of the TLC plate then the spots would dissolve away. If the level of the solvent in the TLC chamber is deeper than the baseline, then the solvent will dissolve the compounds instead of allowing them to ascend the thin layer by capillary action. If this were to occur, in the end you would not see the spots after the plate is developed.

As a drawing exercise, provide the structures of the dyes used in this experiment.**Knowing what you saw in class about intermolecular interactions, circle the groups on each molecule that are liable to interact with the partially acidic, surface Alâ€ OH and Siâ€ OH groups on alumina or silica in neutral solvent.

Fluorescein:

Molecules that are liable to interact with the partially acidic, surface Al- OH and Si- OH

Methylene Blue:

One of the solvents used contained aqueous NaOH. This will generate Alâ€O- and Siâ€O- groups on alumina or silica, and these will be in competition with the solvent for interactions with the analyte. What will this solvent do to the mobility of the dyes?

Although the experiment you performed used the most common chromatography techniques, there are many other types of chromatography. One technique is called ion exchange chromatography, especially useful in

biochemical work. Briefly describe the principle behind ion exchange chromatography and what it can accomplish.

Ion exchange chromatography is a separation technique based on charges. It is used to separate ions and other charged molecules. There are two types of ion exchange chromatography, cation exchange chromatography and anion exchange chromatography. In cation exchange chromatography positively charged molecules are attracted to a negatively charged solid support and in anion exchange chromatography, negatively charged molecules are attracted to a positively charged solid support 4.

In ion exchange chromatography the mobile phase, usually water or an organic solvent, is of low conductivity, which helps in the binding of the molecules 4. As the compound is passed through, like charges repel and elute first and opposite charges attract and elute last. The strength of the interaction is determined by the number and location of the charges on the molecule and on the functional group. By increasing the salt concentration the molecules with the weakest ionic interactions start to elute from the column first 4.

This type of chromatography is essentially important in the separating and isolating carbohydrates. It is also important in separating small inorganic and organic ions 5.