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Foam Separation of Dyes: Enrichment of Minute Target Impurities

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Abstract

The purpose of this study is to separate impurities from dyes using a simple separatory bottle in order to detect to allow for the characterization of these impurities using analytical separation techniques. Foaming agents were used in a glass bottle with a modified cap, to separate a target impurity using the appropriately charged ligand. By running N₂ gas through the solution, the cationic surfactant cetylpyridinium chloride (CPC) and anionic surfactant sodium dodecyl sulfate (SDS) generated foams that separated the anionic dye Orange G and cationic dye Methylene blue, respectively, from a solution containing both dyes. It was found that SDS was able to condense minute quantities of methylene blue from the solution, with high purity. CPC was also able to condense minute quantities of Orange G from the solution, however with less purity. A range of 100nmol to 50μmol of each target dye was tested. For both surfactants, the condensability was shown to increase exponentially as concentration of the target dye decreased. This novel separation method could potentially prove to be a simple, cheap, and effective way to prepare samples and identify components of impurities using HPLC, NMR, and mass spec as well as be used to purify difficult to separate charged compounds.

Keywords

Separatory bottle; dye separation; foam separation; dry foam; high-speed and cost-effective separation; high performance liquid chromatography (HPLC)

1. Introduction

Foam separation has been used as a technique to separate mixtures based on their selective adsorption to a bubble surface. Separations of microparticles and solutes according to their foam affinity have long been carried out using various designs of tubular columns [1–10]. In the tubing space, however, foams tend to form wet foams which carry excess of mother

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liquid around to contaminate the foam fractions. Armstrong et al. have solved this problem using a column filled with glass beads to shed the mother liquid from wet foams to improve the separation [1]. More recently, it has been demonstrated that a simple glass bottle used as a foam separation column can create dry foams to achieve an excellent separation of Rhodamine G and Evans Blue with sodium dodecyl sulfate as a surfactant [2]. This separatory bottle overcomes the primary limitation of previous foam column techniques by creating stable, dry foam, allowing for easier separation of samples. The aim of the present paper is to further investigate the ability of this new simple foaming system for enrichment of minute components from a bulk solution. A variety of combinations of surfactants and sample dyes have been tested. The primary rationale of this experiment was to determine whether the high condensability of minute target component would facilitate the sample preparation for High Performance Liquid Chromatography (HPLC) and other analytical techniques such as NMR and Mass Spectrophotometry. A higher condensability would mean that there is an increased concentration of the sample, therefore allowing for better characterization of impurities. Generally, dyes are purified using countercurrent chromatography and then HPLC, thin layer chromatography, NMR, or mass spec are used to quantify and determine the minor components of these impurities [3]. Previously, Weisz et al. found that countercurrent chromatography could be used for purification of dyes tetrabromotetrachlorofluorescein and phloxine B, followed by HPLC or thin layer chromatography to measure the purity of the product [3]. This is especially important because for identification, larger, uncondensed samples cannot be analyzed using HPLC and other analytical separation methods because of sample absorption. Using this novel separatory bottle technique, however, and the principles of condensability, could prove to be a more efficient, and due to the stability of the dry foam, could result in fewer impurities. This has tremendous implications in the field of cosmetics, food coloring, food additives, etc.

2. Experimental

2.1 Apparatus

Figures 1 and 2 show the design of the present new foam separation systems. In one set of experiments, 2 glass separatory bottles were connected in series. In another set of experiments, a single glass separatory bottle was used. Each bottle (Wheaton Industries, Millville, NJ, USA) has a Teflon lid (NIH Machine Shop) with a pair of flow tubes, one for feeding and the other for collecting foams. The separatory bottle has a 250 mL capacity, and the diameter and the height of the bottle are 6 cm and 13 cm respectively. A magnetic stirrer (Corning Hot Plate Stirrer) was purchased from Corning, NY, USA.

2.2 Reagents

Surfactant ligands such as sodium dodecyl sulfate (SDS) and cetylpyridinium chloride (CPC) were purchased from Sigma Chemicals, St. Louis, MO, USA. Orange G dye was also obtained from Sigma Chemicals, while Methylene Blue dye was obtained from the Hartman-Leddon Company, Philadelphia, PA, USA. Pressured N₂ gas was supplied by Roberts Oxygen, Rockville, MD, USA.

2.3 Preparation of surfactant solution and sample solution

A stock solution of 1mM CPC was prepared by dissolving 358 mg of CPC in 1 L of water and prepared for continual use.

1 mM dye solutions were similarly created for continual use. A stock solution of 1mM Methylene Blue was prepared by dissolving 82 mg of Methylene Blue in 250 mL of water. A stock solution of 1mM Orange G was prepared by dissolving 113 mg of Orange G in 250 mL of water. For both dyes, 100 mL of 500 μ M, 100 μ M, 50 μ M, 10 μ M, 5 μ M, and 1 μ M solutions were prepared, making a total of 12 solutions that were tested.

2.4 Procedure for Foam Separation

57mg SDS was added to a bottle. The bottle was connected, at one end, to a supply of compressed N₂ gas. The other end was the foam output that was collected through fractionation.

50 mL of 1mM Methylene Blue was diluted with water to 100 mL of 500 μ M Methylene Blue and added to the bottle. A constant 5 mg of the opposing charged dye, Orange G was added to the bottle at each concentration of the sample solution. The bottle was closed tightly to avoid the leakage of any gas or foam. The N₂ gas valve was then opened and set to 60 psi. The resulting foam from the bottle was collected into a large test tube. These steps were repeated for the other concentrations of dye (10 mL, 5 mL, etc. diluted to 100 mL).

For the next set, about 300 mL of 1mM CPC was added to a bottle. The bottle was connected, at one end, to a supply of compressed N₂ gas. The other end was connected to a second bottle. 50 mL of 1mM Orange G was diluted with water (1:1) making a total volume of 100 mL. This dilution was then added to bottle 2. About 500 mg, or 3 pellets, of sodium hydroxide was added to each sample (bottle 2) to aid dissolution. A constant 5 mg of the opposing charged dye, Methylene Blue was added to bottle 2 at each concentration of the sample solution. Bottle 2 was then connected to bottle 1, and at the other end, to a large test tube. Both bottles were closed tightly as to not leak any gas. The N₂ gas valve was then opened and set to 60 psi. The foam from bottle 2 was collected into the large test tube. These steps were repeated for the other concentrations of dye (10 mL, 5 mL, etc. diluted to 100 mL).

2.5 Analysis of foam fractions

For determination of the condensability by volume, the initial (100 mL) and final volumes of the test tube collection were measured.

The ratio of the initial volume to the final volume is the condensability (by volume) of the dye at that concentration (Equation 1).

$$\text{Condensability (by volume)} = \frac{\text{Initial Volume}}{\text{Final Volume}} \quad (1)$$

Additionally, condensability was also measured by concentration by measuring the absorbances of the sample initially and the test tube collection. The ratio of the initial sample absorbance to the final test tube collection absorbance is the condensability (by concentration) of the dye at that concentration (Equation 2).

$$\text{Condensability (concentration)} = \frac{\text{Initial Sample Absorbance}}{\text{Final Sample Absorbance}} \quad (2)$$

For measurement of the Orange G/CPC combination, the absorbances were measured at 490 nm. For measurement of the Methylene Blue/SDS combination, the absorbances were measured at 555 nm. These respective wavelengths were determined using an absorbance curve.

3. Results and Discussion

3.1 Condensability by Volume

As the primary aim of this foam separation was to obtain a sample that could be utilized for analytical separation, the concentration of the samples into a smaller volume was the primary goal, with purification as a secondary goal. When condensability by volume was calculated for both the SDS/Methylene Blue and the CDC/Orange G, it was noted that there was an indirect relationship between the concentration and condensability. With increasing initial concentrations, there was a decrease in condensability (Figures 3 and 4). Furthermore, volume condensability of up to 200x was observed at 1 μ M, and as high as 13x was noted at higher concentrations i.e. 500 μ M. While all initial volumes were 100 mL, final collection volumes ranged from 0.5 mL to 7.5 mL.

3.2 Condensability by Concentration

The condensability (by concentration), measured by the ratio of final absorbance to initial absorbance (Equation 2), for SDS/Methylene Blue showed a similar indirect trend to that of the condensability by volume: condensability exponentially decreased as concentration increased (Figure 6). However, the condensability (by concentration) for CPC/Orange G did not observe the same trend (Figure 5), and a relationship between concentration and condensability was not noted. Initial concentrations ranged from 1 μ M to 500 μ M, while final concentrations ranged from 50 μ M to 3mM.

3.3 Discussion

It was found that the 1 bottle setup works better for SDS, and the 2 bottle setup works best for CPC, possibly a result of micellization, phase separation. The critical micelle concentration (CMC), the concentration above which surfactants will form micelles, of SDS is around 8mM, while the CMC of CPC is around 1mM. It is possible that with the 1 bottle setup, 1mM CPC would form micelles, decreasing its ability to bind to Orange G molecules, inhibiting separation. A 2-bottle setup would avoid this since the CPC is introduced into the second bottle at a slow pace, starting with no initial CPC and consistently staying under the CMC. SDS avoids this problem altogether - 2mM solutions are created, therefore remaining

under the 8mM CMC. The high condensability (by volume) of low initial concentration solutions in some sense is arbitrary, depending in part on the initial volume. However, it is observed that at lower concentrations and higher volumes, condensability (by volume) is higher. Additionally, this is optimal for HPLC application as samples would likely be prepared at higher initial volumes and lower initial concentrations.

The condensability (by concentration) of SDS/Methylene Blue showed similar results to the condensability (by volume). This could be an indication that the collection was both concentrated and pure, meaning the foam collected only the counterion to which it was attracted. The condensability of CPC/Orange G did not demonstrate this trend. This could be an indication that the collection, although condensed (Figure 5), was not as pure as the collection formed from SDS/Methylene Blue. It is therefore concluded that CPC is not as effective as SDS in purifying, although it results in a similar level of condensability.

4. Conclusions

The use of a bottle in the foam separation technique is a cheap, yet viable method for facilitating presentation of a concentrated and condensed sample for use in analytical separation methods. This remains true for both CPC and SDS as foaming affinity ligands. When using SDS as the surfactant ligand, the collection was both concentrated and pure, further facilitating sample preparation for use in HPLC. CPC as a positively charged foaming ligand, worked moderately well, but still collected more mother liquid than desired. In trying to find viable positively charged surfactant ligands, benzethonium chloride and benzalkonium chloride were dismissed because of their poor ability to condense or purify. Possible cationic foaming ligands could include cetrimonium bromide and carbethopendecinium bromide. Apart from its utilization for analytical separation sample preparation, the separatory bottle can be used to separate difficult to separate, charged compounds which could have broader potential applications such as separation of enantiomers using foam-producing chiral selectors and separation of oppositely charged ionic compounds, or purification of charged compounds. Further future investigations can be done to identify positively charged ligands that more effectively collect the counterion without an excess of mother liquid, and to see if this foam separation method is viable for biological samples. Additionally, future experiments could look at separating uncharged targets from solution. Finally, we hope to collaborate with other researchers to utilize HPLC, Mass Spec, thin layer CCC, and other analytical methods to determine the components of the impurities that we have managed to isolate using our separatory bottle technique.

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Figure 1:
Photograph of the 1 bottle setup involving separation of Methylene Blue using SDS.

Figure 2:
Photograph of the 2 bottle setup involving separation of Orange G using CPC.

Figure 3:

Condensability (by volume) of Orange G with 100mL 1mM CPC. Lower concentrations of the target yield higher condensability, as they require less volume in which to elute.

Figure 4: Condensability (by volume) of Methylene Blue with 100mL 2mM SDS. The same trend as that of Figure 1 is noted here: lower concentrations of the target yield higher condensability, as they require less volume in which to elute.

Figure 5:
Condensability (by concentration) of Orange G with 100mL 1mM CPC. The trend seen in Figures 1 and 2 is not observed. This is an indication that the sample may not be pure, although it is condensed (Figure 1).

Figure 6:

Condensability (by concentration) of Methylene blue with 100mL 2mM SDS. A similar trend to Figures 1 and 2 is noted here. This is an indication that the sample is both condensed and pure.

Table 1.

Table of raw data summarizing initial concentration, final volume, condensation, final absorption, and final concentration which were used to determine the correlations between initial concentrations and condensability

Condensability of MB w/ SDS at 100mL Starting Liquid Volume							
Initial Mass (mg)	Initial Conc. (mM)	Final Volume (mL)	Condens. (Vol)	Starting Abs (A_{555})	Final Abs (A_{555})	Final Conc. (mM)	Condens. (Abs)
						Based on Abs.	
0.0328	0.00	1.00	100.00	0.01	0.21	0.00	35.33
0.164	0.01	4.00	25.00	0.03	0.35	0.01	13.42
0.328	0.01	5.00	20.00	0.05	0.51	0.01	9.60
1.64	0.05	8.00	12.50	0.14	0.87	0.05	6.23
3.28	0.10	12.50	8.00	0.18	1.01	0.10	5.50
16.4	0.50	25.00	4.00	1.08	1.53	0.50	1.41
Condensability of Orange G w/ CPC at 100mL Starting Liquid Volume							
Initial Mass (mg)	Initial Conc. (mM)	Final Volume (mL)	Condens. (Vol)	Starting Abs (A_{490})	Final Abs (A_{490})	Final Conc. (mM)	Condens. (Abs)
						Based on Abs.	
0.04	0.00	0.50	200.00	0.02	0.10	0.01	5.76
0.18	0.01	1.00	100.00	0.01	0.06	0.02	4.87
0.36	0.01	2.00	50.00	0.02	0.16	0.10	9.69
1.79	0.05	4.00	25.00	0.03	0.28	0.50	10.02
3.58	0.10	4.00	25.00	0.05	0.57	1.09	10.88
17.90	0.50	7.00	14.29	0.23	1.41	3.02	6.04