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Foam separation of Rhodamine-G and Evans Blue using a simple separatory bottle system

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Abstract

A simple separatory glass bottle was used to improve separation effectiveness and cost efficiency while simultaneously creating a simpler system for separating biological compounds. Additionally, it was important to develop a scalable separation method so this would be applicable to both analytical and preparative separations. Compared to conventional foam separation methods, this method easily forms stable dry foam which ensures high purity of yielded fractions. A negatively charged surfactant, sodium dodecyl sulfate (SDS), was used as the ligand to carry a positively charged Rhodamine-G, leaving a negatively charged Evans Blue in the bottle. The performance of the separatory bottle was tested for separating Rhodamine-G from Evans Blue with sample sizes ranged from 1 to 12 mg in preparative separations and 1 to 20 μ g in analytical separations under optimum conditions. These conditions including N₂ gas pressure, spinning speed of contents with a magnetic stirrer, concentration of the ligand, volume of the solvent, and concentration of the sample, were all modified and optimized. Based on the calculations at their peak absorbances, Rhodamine-G and Evans Blue were efficiently separated in times ranging from 1 hour to 3 hours, depending on sample volume. Optimal conditions were found to be 60 psi N₂ pressure and 2 mM SDS for the affinity ligand. This novel separation method will allow for rapid separation of biological compounds while simultaneously being scalable and cost effective.

Keywords

Separatory bottle; dye separation; foam separation; dry foam; high-speed and cost-effective separation

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1. Introduction

Foam has been used for the separation of various samples based on their foam affinities [1–10]. The use of ionic surfactants as ligands allows for the bioseparation of samples according to the differential charge affinity. Foam has two different forms, i.e. wet foam and dry foam. The wet foam, formed at the lower portion of the column, is small and round in shape, holding a large amount of excess liquid between the portions. As the foam moves upward, it coalesces and grows larger, shedding the surrounding liquid and becoming dry foam, which is composed of a broad thin membrane. A major limitation of foam separation is that it is difficult to make and maintain the stable dry foam. A number of methods using tubular columns have been developed, to generate stable dry foam, which tolerate high pressures and separate biological compounds effectively. Stable dry foam, however, is difficult to obtain especially using a narrow tubular column, and the primary limitations with most methods that attempt to extract dry foam are that they are not cost-effective, cannot separate in large quantities, and often result in contamination for simpler columns. Previously, Armstrong et al. [1] used adsorptive bubble techniques using a glass foaming chamber with glass beads to create dry foam inside the column. In order to overcome these limitations, we have developed a new system using a separatory bottle which is cost effective, scalable, efficient, and can separate a range of quantities of samples, from micrograms to milligrams, therefore allowing for it to have broad applications for analytical and preparative separations.

2. Experimental

2.1 Apparatus

The separatory bottle used in the present studies is a 250 mL capacity glass bottle purchased from Wheaton Industries, Millville, New Jersey, USA (ordinary glass bottles of similar sizes can be used). We made a Teflon plug which holds one gas inlet and one foam outlet (Teflon tubing 0.85 mm ID, SW20, Zeus Industrial Products, Orangeburg, SC, USA) as shown in Figure 1. The diameter and height of the bottle are 6 cm and 13 cm respectively. The bottle is protected by wrapping it with a transparent adhesive tape for safety (the bottle was tested against 100 psi before wrapping). A magnetic stirrer (Corning Hot Plate Stirrer) was purchased from Corning, NY, USA.

2.2 Reagents

Sodium Dodecyl Sulfate (SDS) was purchased from Sigma Chemicals, St. Louis, MO, USA. Rhodamine-G was purchased from the National Aniline Division Pharmaceutical Laboratories, New York, NY, USA. Evans Blue was also purchased from Sigma Chemicals. Pressured N₂ gas was supplied by Roberts Oxygen, Rockville, MD, USA.

2.3 Preparation of stock sample solution and sample solutions

The stock sample solution was prepared by dissolving 100 mg of Rhodamine-G and 100 mg of Evans Blue in 100 mL of 1 mM SDS. For the preparative separation, sample solutions were made by diluting the stock solution with 10 times the volume of the 2 mM SDS, making 12 solutions (10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, and 120 mL) which were

tested. For analytical separations, 1 μL , 5 μL , 10 μL , 15 μL , and 20 μL of the stock sample solutions were each added to 100 mL of 2 mM SDS solution containing 100 mg of Evans Blue.

2.4 Procedure for preparative separation

The separatory bottle containing the sample solution was placed on a magnetic mixer to constantly mix the contents while the N_2 gas was introduced into the bottle at 60 psi. Then, the foam from the collection line was fractionated at 1 to 2 mL into test tubes.

Additionally, different parameters were tested with varying gas pressures, concentrations of SDS, sample volume, and dilution of sample. Once the optimum parameters were determined, all runs were conducted at those parameters, changing only the sample volume.

Volumes ranging from 11 mL (10 mL SDS + 1 mL stock sample solution) to 220 mL (200 mL SDS + 20 mL stock sample solution) were tested for the preparative separation. Gas pressures of 40, 60, and 80 psi were tested. Both 1 and 2 mM SDS solvent systems were run to see which was the optimal concentration.

2.5 Procedure for analytical separation

For analytical separations, a 5 mg % Evans Blue solution was made by adding 5 mg of Evans Blue to 100 mL of 2 mM SDS, to which 1 to 20 μL of stock sample solution was added in the separatory bottle. The bottle contents were mixed with a magnetic mixer while nitrogen gas was injected into the inlet of the bottle at 60 psi. Sample sizes ranging from 1 to 20 μg were tested to determine whether this system could separate such small amounts of sample.

2.6 Analysis of foam fractions

In order to determine which parameters were optimal, and to identify whether, quantitatively, the separation had occurred, the absorbance of pure samples of Rhodamine-G was measured using the ThermoFisher (Waltham, MA, USA) spectrophotometer to determine the ratio between the absorbances at 480 nm (peak maximum of Rhodamine-G) and at 620 nm (peak maximum of Evans Blue). The absorbances were 0.073 and 0.024, respectively, and the ratio between these two absorbances of the pure Rhodamine-G was found to be 3.04. Then, the following equation was created to evaluate the purity of the separated Rhodamine-G:

$$\Delta A = \left(\frac{A_{480}}{3.04} \right) - A_{620}, \quad (\text{Eq. 1})$$

where A_{480} and A_{620} indicate the absorbance of the foam fraction measured at 480 nm and 620 nm, respectively. If the difference (ΔA) is approximately equal to 0, the separation has effectively occurred without contamination.

3. Results and Discussion

3.1 The optimum length of the foam receiving tube

The optimum length of the foam receiving tube from the Teflon plug hanging down into the separatory bottle was determined. The lengths of 0 cm, 5 cm, and 7 cm were tested for the preparative separations, and it was found that there were no significant differences in separations. For preparative use, therefore, and for simplicity, it is easiest to make the tube length of the outlet 0 cm. When comparing tube lengths for the analytical samples, the separation was optimal at a tube length of 0 cm. When tube length was tested at 5 or 7 cm, the separation was ineffective.

3.2 Analysis of foam fractions

Near complete purification was attained for all volumes ranging from 21 mL to 240 mL as seen in Figures 2a and 2b. This demonstrates that the distance from the liquid to the plug of the bottle needs to be only a few centimeters for the foam to separate the dyes. Moreover, this method also shows that the foam is stable when using a solvent system of 2 mM SDS, at a gas pressure of 60 psi, and a dilution of stock sample solution at 1:10 for each run. The primary limitation of this study is that manual fractionation has to be done, as no machine exists thus far to fractionate foam eluents. Additionally, as the sample volumes began to increase, as expected, the times for the runs also increase. One way to overcome this limitation would be to increase the gas pressure, but if the gas pressure is increased excessively, the foam will not be stable and the dyes will exit the outlet rapidly before complete purification has occurred. Analytical separation shown in Figure 3 and Electronic Supplementary Material 1 demonstrated that some amounts of Rhodamine-G tends to remain on the inside the inner wall of the glass bottle near the outlet, resulting in negative

A values calculated using Eq. I. This was true for small sample sizes except for when the sample size was increased to 20 μ L (containing 20 μ g of Rhodamine-G in 100 mL of the sample solution). This problem will be addressed as a part of future investigations.

Overall, however, this novel method using the separatory bottle is cost effective, creates stable dry foam effectively, is efficient in complete preparative purification (Figure 3), and has broad applications in the industrial field as large volumes of the mixtures can be separated easily. Most importantly, this bottle was able to separate sample sizes ranging from 20 μ g to 12 mg of Rhodamine-G. This demonstrates the scalability of this method.

3.3 Measurement of condensability of the method in preparative separation

In analytical separation the sample volume should be kept minimal to obtain sharp peaks of the target compounds. Therefore, when the the target compound(s) concentration is low, it is necessary to condense the target compound(s) in the sample solution. Therefore, the condensability of the method is an important parameter in this separation method. This parameter is determined by calculating the total sample volume divided by the volume of the total fractions obtained in the preparative separation. As shown in Electronic Supplementary Material 2, the volume eluted out was also calculated by measuring the volume remaining within the bottle and subtracting it from the total initial volume. The volumes ranged from 5 to 11 times increase in condensation. The original sample volume ranged from 160 mL to

220 mL and the volume increased as the sample size was increased. Therefore, this method may be efficiently used for the sample preparation (Table 1).

4. Conclusions

The separatory bottle method is a new foam separation method that has not been reported before. In the past, foam separation has been carried out using rather complex tubular glass columns. Compared with these conventional column designs, our system is so simple that any researcher can easily build and use it at almost no cost. Despite its simplicity, the present system has broad potential applications such as the separation of ionic compounds using foam-producing counter-ions as an affinity ligand, fractionation of foaming samples such as saponins and peptides, and the separation of enantiomers using foam-producing chiral selectors like bovine serum albumin and β -cyclodextrin derivatives. Continuous sample feeding is made by adding a sample feed tube to the Teflon plug. The separation may be improved by connecting multiple separatory bottles.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

1. A simple separatory bottle provides efficient separation of dyes
2. The method is scalable, allowing for both preparative and analytical separations.
3. This method is cost effective and easily accessible.

Figure 1. Diagram of the separatory bottle with the foam outlet, gas inlet, stirrer, and Teflon plug marked as indicated.

Figure 2.

a) Photograph of the separatory bottle during the separation demonstrating how the Rhodamine-G separates by moving to the top in the foam before reaching the outlet b) Photograph of the separatory bottle after the separation showing how only the Evans Blue remains within the bottle.

Figure 3.

This figure is a compilation of the data done for the analytical experiments, demonstrating that after calculating the ratios of the absorbances, we identified that for all the sample sizes that were run, there was no contamination in the separation. This demonstrates full separation of the two dyes.

Table 1

Purity of rhodamine G separated from the mixture using foam separation.

Sample volume in ml.	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1*	3	.0482	.02500	.01444	-.0139	.1103	.03	.08
2	6	-.0009	.00381	.00156	-.0049	.0031	-.01	.00
3	4	.0045	.00930	.00465	-.0104	.0193	.00	.02
4	5	.0039	.00851	.00381	-.0066	.0145	.00	.02
5*	9	-.0011	.00237	.00079	-.0029	.0007	.00	.00
6	16	-.0072	.00253	.00063	-.0086	-.0059	-.01	.00
7	10	-.0026	.00233	.00074	-.0043	-.0009	-.01	.00
8	5	-.0045	.00414	.00185	-.0096	.0007	-.01	.00
9	5	-.0012	.00254	.00114	-.0043	.0020	.00	.00
10	5	-.0053	.00064	.00029	-.0061	-.0045	-.01	.00
11	5	-.0042	.00280	.00125	-.0077	-.0008	-.01	.00
12	8	-.0092	.00201	.00071	-.0108	-.0075	-.01	-.01

N refers to the number of fractions assayed from each run.

All means are near zero as expected with a pure fraction. Impurities result in values closer to 1.

* Were repeated in at least 2 biological replicates.