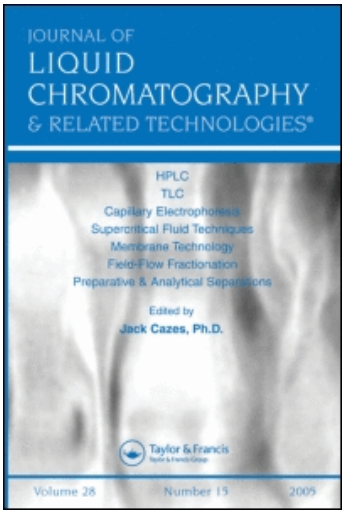

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FOAM COUNTERCURRENT CHROMATOGRAPHY ON VARIOUS TEST SAMPLES AND THE EFFECTS OF ADDITIVES ON FOAM AFFINITY

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ABSTRACT

Foam countercurrent chromatography has been applied to various samples using sodium dodecyl sulfate (SDS), cetyl pyridinium chloride (CPC), and polyoxyethylene-23-lauryl ether (POELE) as foaming reagents. Overall results clearly indicate that the positively charged samples collected with SDS foam and the negatively charged samples with CPC. None of the applied samples showed affinity to POELE. Addition of hydrochloric acid or sodium hydroxide (each at 0.01N concentration) to the surfactant solution effectively reduced the foam affinity of CPC or SDS, respectively, while NaCl at a high concentration of 0.1M showed similar inhibitory action to both surfactants. Among biological test samples, indole-3-acetic acid and abscisic acid were collected with CPC and bovine insulin with SDS foam.

INTRODUCTION:

A recently developed countercurrent chromatography method utilizes a combination of the coil orientation and the planetary motion of the apparatus (1). Both produce a countercurrent flow of two solvent phases. Two phases can be simultaneously eluted through the column in opposite directions while the sample solution is continuously fed through at the middle portion of the column. As a result, a continuous separation of a sample is possible (2).

Unlike other methods of chromatography, the countercurrent chromatography system does not utilize a solid support (3). Thus, countercurrent chromatography is free from all complications arising from solid supports. Adsorptive loss, denaturation, and contamination of samples are minimized. This method and its apparatus form the basis of a relatively new separation technique called foam countercurrent chromatography.

Foam countercurrent chromatography relates to the system of dual countercurrent chromatography (2). The foam countercurrent chromatography method can be applied to numerous samples having foam affinity. Foam affinity can be classified into two categories. One, the direct affinity to the gas-liquid interface, and two, the affinity to the foam producing carrier. Samples, such as detergents and other foam producing substances, can be introduced through the sample feed line without special treatment because they have affinity to the gas-liquid interface. Samples which lack direct affinity to the gas-liquid interface can be indirectly adsorbed to the foam if they have an affinity to the foam producing agents.

Although the foam separation method has been widely used over the past sixty years (4), the method has remained rather primitive and inefficient. In the past, various devices have been developed for performing foam separation. These devices utilized tubular columns where the foam was generated by introducing the gas phase at the bottom of the column. Under the unit gravitational field, the foam moved spontaneously upwards toward the top of the column. Although various mixing devices such as bafflers, solid beads, and rotary mixers are used for improving the results, the use of the short column under the weak gravitational field limits the efficiency of these systems. The results of foam countercurrent chromatography suggest that this method would drastically improve the separation time and efficiency of conventional methods to become an essential tool for scientific research in laboratories in the near future.

Recently, the potential capability of foam countercurrent chromatography has been successfully demonstrated on the separation of rhodamine B and Evans blue with sodium dodecyl sulfate as a surfactant and also on protein separation with a phosphate buffer solution (1). The present paper deals with the foam affinity of various compounds to three typical surfactants (cationic, anionic, and nonionic) and the effects of various additives to the surfactant solution.

APPARATUS:

The apparatus used for this separation method is a multilayer flow-through coil planet centrifuge which produces a synchronous

planetary motion of the gear driven column holder (Figure 1). The gear driven column holder revolves around the central axis of the apparatus and simultaneously rotates about its own axis at the same angular velocity. The planetary motion induces a countercurrent flow of gas and liquid through a coiled column coaxially mounted about the holder. The column design in foam separation is schematically illustrated in figure 2. The coiled column, consisting of a ten meter long piece of 2.6 mm i.d. PTFE (polytetrafluoroethylene) tubing with a fifty milliliter capacity, is equipped with five flow channels: a gas feed line and a liquid collection line at one end which is called the head, a liquid feed line and a gas or foam collection line at the other end (called the tail), and a sample feed line at the middle portion of the column. Foam separation begins by the simultaneous introduction of the surfactant solution through the tail (pump rate is 214 ml/hr) and nitrogen gas (from a gas cylinder at 80 p.s.i.) through the head into the rotating coil which establishes a steady state hydrodynamic equilibrium. This equilibrium moves the liquid stream toward the head and the gas or foaming stream towards the tail. The sample mixture which is introduced through the sample feed line by a syringe is separated according to its affinity to the foam. Any compound having foam affinity is carried with the foaming stream toward the tail and dispensed through the foam outlet (foam collection line). Other materials are carried with the liquid stream in the opposite direction toward the head and eluted through the liquid outlet. The liquid flow through the liquid collection

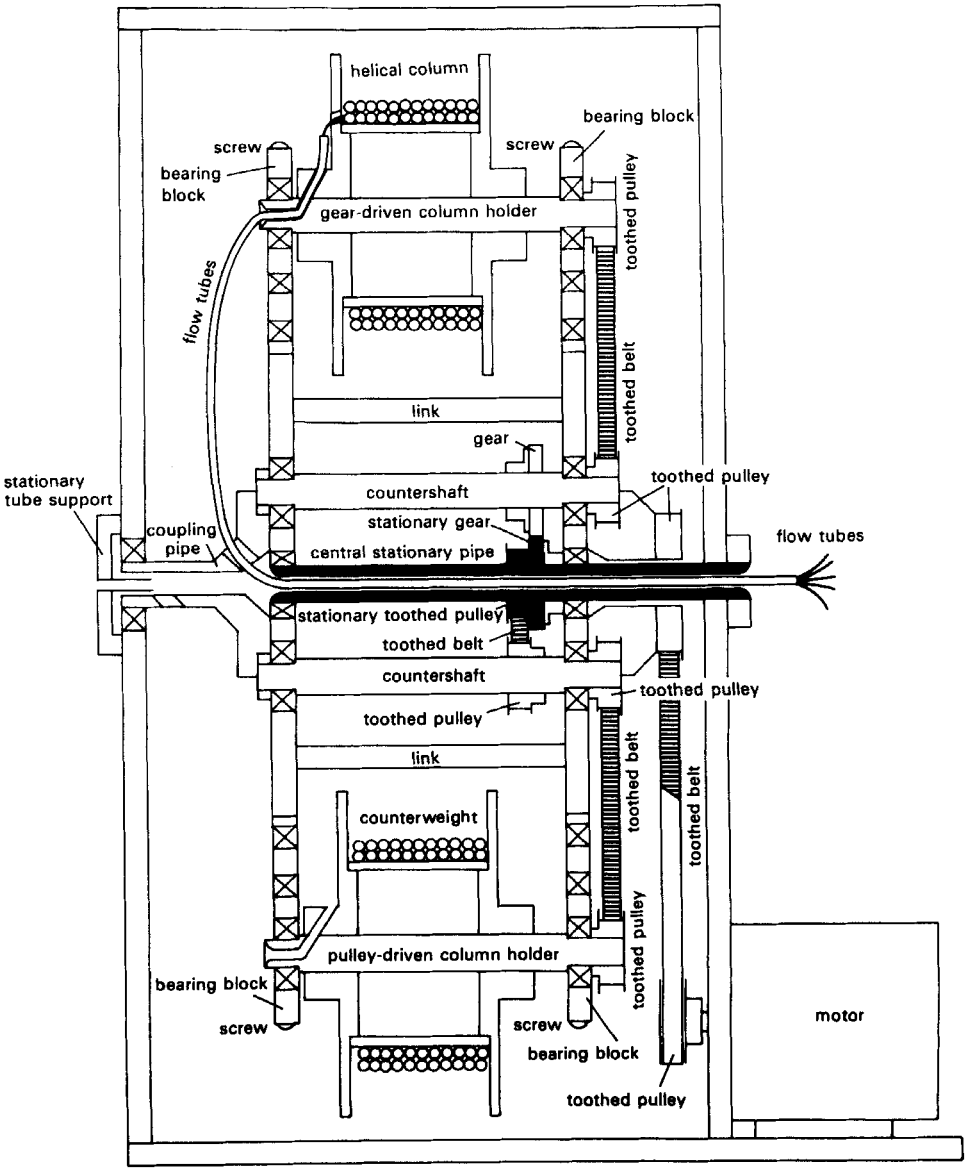


Figure 1: Cross sectional view of apparatus

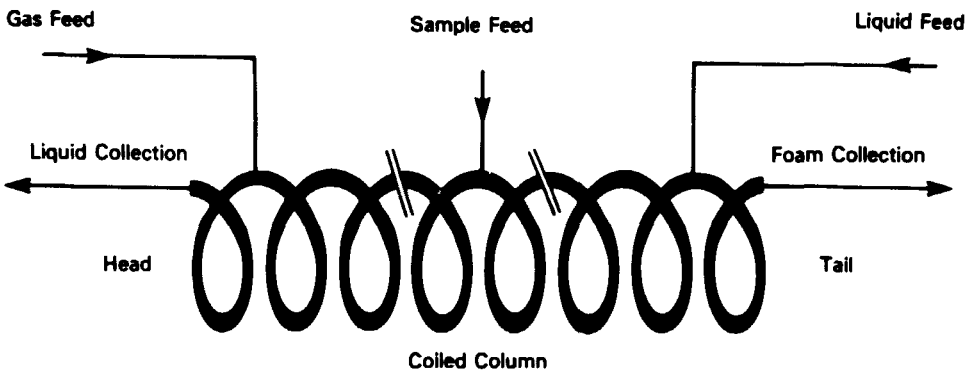


Figure 2: Column design of foam countercurrent chromatography

line is regulated by a needle valve (Model SS-1SG; Potomac Valve and Fitting Company, Rockville, MD) while the foam is left undisturbed, directly open to the air.

EXPERIMENTAL:

Reagents:

In the present studies, sodium dodecyl sulfate (Sigma Chemical Company, St. Louis, MO) which is anionic, polyoxyethylene-23-lauryl ether (Sigma Chemical Company) which is non-ionic, and cetyl pyridinium chloride (Sigma Chemical Company) which is cationic were used as foaming reagents. Each surfactant was used with the optimum concentration previously determined. Methanol (Burdick and Jackson

Laboratories, Muskegon, MI), sodium chloride (J.T. Baker Chemical Company, Phillipsburg, N.J.), hydrochloric acid (J.T. Baker Chemical Company), and sodium hydroxide (J.T. Baker Chemical Company) were added to these surfactants to observe their effects upon the separation of various colored samples. The above chemicals are all reagent grade. Colored samples were chosen as test samples, including various DNP amino acids like DNP-leucine, acidic dyes such as methyl orange, and basic samples including methylene blue chloride (all supplied by Sigma Chemical Company). Pigments were of technical grade and DNP amino acids were of reagent grade. Noncolored samples included ones such as ATP, bovine insulin, and indole-3-acetic acid. All of these samples are of reagent grade (all supplied by Sigma Chemical Company).

Procedures:

Preliminary test runs were performed with the colored samples of methylene blue and DNP-leucine. The introduction of 0.5 ml of sample through the sample feed line was immediately followed by 1.0 ml of surfactant solution. Foam from the foam collection line and the effluent from the liquid outlet were collected in separate 10 ml-capacity graduated cylinders. Results were recorded by observing the color of the eluates. These procedures were continued for each colored sample tested. Additives, such as methanol and sodium chloride, were simply incorporated into the surfactant solution.

Noncolored samples such as nucleotides, proteins, and plant hormones were introduced into the system in a similar manner. One-half milliliter (0.5 ml) of sample solution was injected into the middle portion of the coil followed by 1.0 ml of surfactant solution. The eluate from the outlet of each collection line was manually fractionated into test tubes at 30 second intervals. Absorbance was measured at suitable wavelengths for each sample with a Zeiss spectrophotometer.

RESULTS AND DISCUSSION:

Sodium dodecyl sulfate, polyoxyethylene-23-lauryl ether, and cetyl pyridinium chloride were used as surfactants in investigating the effects of electrical charges on the foam affinity of various compounds.

Figure 3 illustrates two sets of chromatograms obtained by foam separation of a methylene blue and DNP-leucine mixture with sodium dodecyl sulfate (SDS) (top) and cetyl pyridinium chloride (CPC) (bottom). In each chromatogram, the ordinate indicates absorbance values measured at two wavelengths, 430 nm for DNP-leucine (●-●) and 620 nm for methylene blue (▲-▲), and the abscissa elution time in minutes as indicated. When the sample mixture was introduced with the anionic SDS surfactant, the positively charged methylene blue adsorbed to the foam and quickly eluted through the foam collection line (top, right) while the negatively charged DNP-leucine was carried with the liquid stream

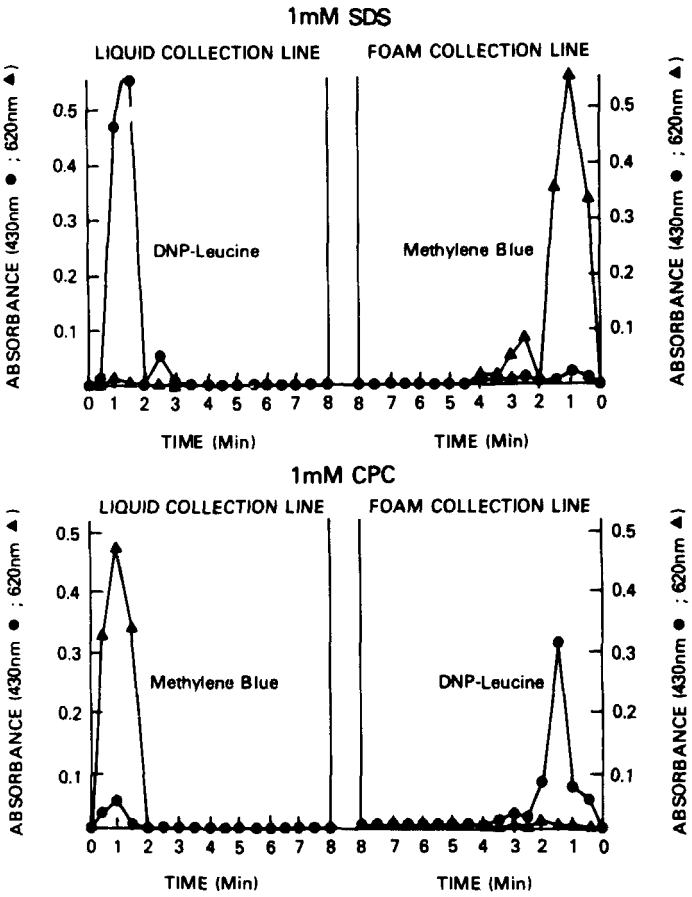
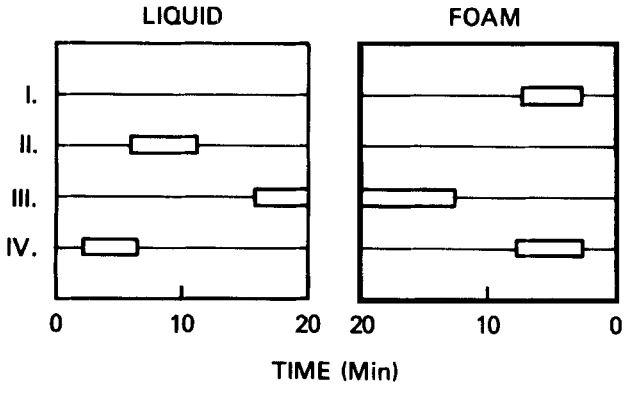


Figure 3: Separation of methylene blue and DNP-leucine with sodium dodecyl sulfate (SDS) (top) and cetyl pyridinium chloride (CPC) (bottom): absorbance values measured at two different wavelengths--430 nm for DNP-leucine and 620 nm for methylene blue (located on ordinate). Time measured along abscissa. With SDS as the surfactant, positively charged methylene blue eluted through foam line while negatively charged DNP-leucine carried with liquid stream. DNP-leucine eluted through foam line and methylene blue carried with liquid line when CPC was used as the surfactant.

in the opposite direction and eluted through the liquid collection line (top, left). Similarly, when the same sample mixture was eluted with the cationic CPC surfactant, the negatively charged DNP-leucine was entirely eluted through the foam collection line (bottom, right) and the positively charged methylene blue through the liquid collection line (bottom, left). (NOTE: The minor absorbance observed below the major peak indicates the absorbance of the same compound at the corresponding wavelengths.) Reproducibility of the above foam separation was examined by eluting each dye separately which yielded a similar peak at the corresponding locations.

Figures 4 I-IV diagrammatically illustrate four typical elution patterns obtained from various colored samples. In each set of chromatograms, colored bands eluted through the liquid collection line are drawn as a bar on the left side with a time scale recorded at the bottom; the colored foam fractions are similarly drawn on the right side. In this way, early eluted fractions either from liquid or foam collection lines are seen near the respective margin of the diagram while later eluted fractions are located near the center of the diagram.

Chromatogram I shows a typical elution pattern of a strongly foam active compound which is quickly eluted out through the foam collection line. Chromatogram II is a typical elution profile of a compound which lacks foam affinity and therefore is quickly eluted through the liquid collection line. When the compound has a moderate degree of foam affinity, it will remain in the column for a longer period of time and is subjected to additional mixing which



Figures 4 I-IV: Illustration of four typical elution profiles. Colored bands eluted through liquid collection line are drawn as a bar on the left side. Similarly, material in foam line drawn on the right side of the diagram. Time scale is located at the bottom of the figure. Early eluted fractions appear near their respective margins of the figure while later fractions are seen near the center.

causes extensive broadening of the sample band. This results in elution of the sample through both foam and liquid collection lines as shown in chromatogram III. Some samples produce elution patterns as shown in chromatogram IV. This may indicate that the sample contains two different compounds; one is foam active and the other is inactive, each separately eluted through their respective collection line.

Screening tests for foam affinity were performed on various samples such as DNP amino acids, and acidic and basic dyes using sodium dodecyl sulfate (SDS), cetyl pyridinium chloride (CPC), and polyoxyethylene-23-lauryl ether (POELE) as foam producing reagents with or without additives in the surfactant solution. Figure 5 summarizes the results obtained with SDS and CPC as surfactants

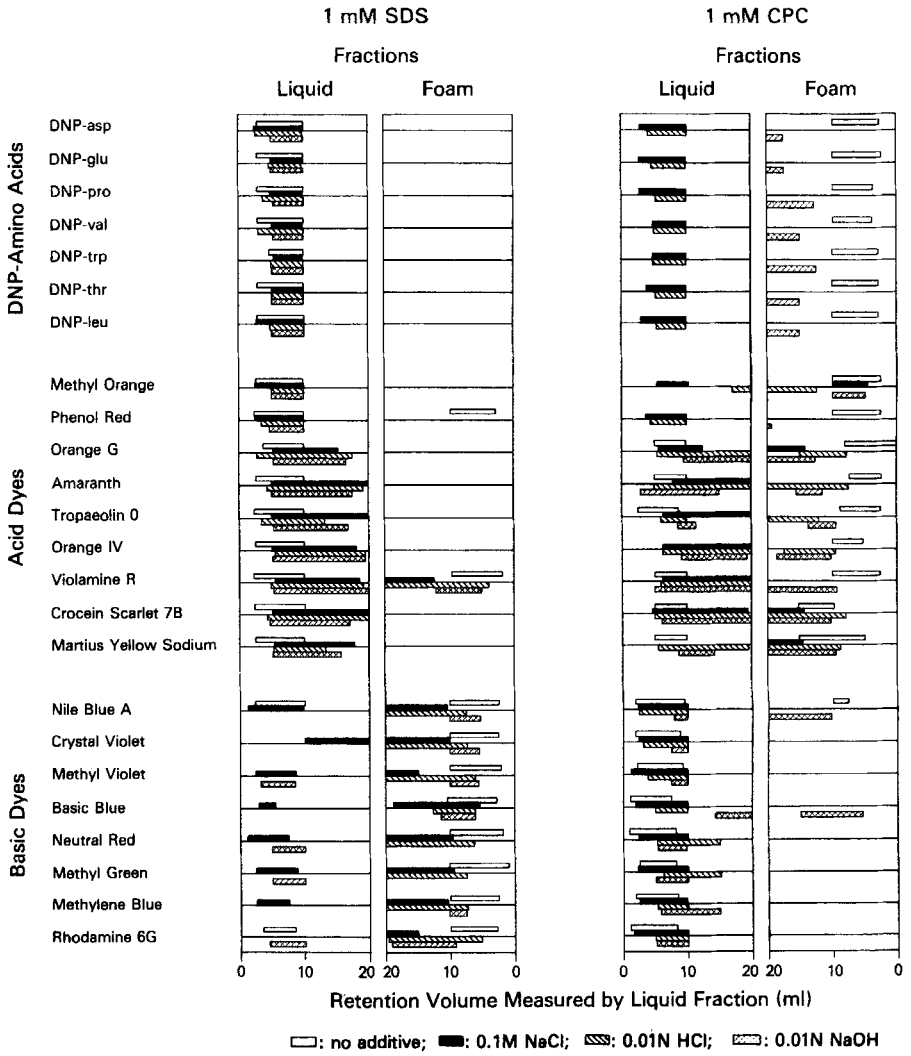


Figure 5: Shows the separation of test samples and the effects of various additives on foam affinity. Clear bars represent results without additives in surfactant solution. The effects of 0.1M NaCl (neutral salt) are shown by solid bars. Sodium chloride inhibited CPC foam affinity of DNP amino acids and acid dyes and also inhibited SDS foam affinity of basic dyes. Hydrochloric acid (shown as striped bars) (0.01N) suppressed CPC foam affinity of acid dyes; it slightly inhibited SDS foam affinity of basic dyes as well. Sodium hydroxide (checked bars) (0.01N) produced inconsistent results: it effected SDS foam affinity of basic dyes like neutral red and methyl green and it effected CPC foam affinity of DNP amino acids and acid dyes. Methanol is not pictured because no significant effect observed.

according to the format illustrated in Figures 4 I-IV. POELE was omitted from the figure because none of the samples tested showed foam affinity. Among all samples tested, DNP amino acids (reagent grade) produced a clear cut behavior while both acidic and basic dyes (technical grade) gave some irregular responses, probably due to the heterogeneity of the sample.

Results obtained with a surfactant solution containing no additives (shown as clear bars in Figure 5) were essentially the same as those obtained with methylene blue and DNP-leucine previously described. Both DNP amino acids and acid dyes showed foam affinity to CPC while basic dyes showed affinity to SDS. Some exceptions were observed in both acidic and basic dyes: phenol red and violamine R (acid dyes) were partly carried with the SDS foam and Nile blue A (basic dye) with the CPC foam. None of the samples showed foam affinity to POELE as mentioned above.

The effects of various additives on the foam affinity of the above samples (Figure 5) were studied by using various chemicals such as sodium chloride (neutral salt), hydrochloric acid (acid), sodium hydroxide (alkali), and methanol (organic solvent). Among these, 0.01M NaCl and 10% methanol failed to produce any significant change in the foam affinity for the above samples and therefore were eliminated from the figure. When NaCl was used in a higher concentration at 0.1 M, it produced highly significant effects on the foam affinity of both surfactants as indicated by the solid bars in Figure 5 (i.e., strong inhibitory effects on CPC foam affinity of DNP amino acids and acid dyes and on SDS foam affinity of basic dyes were observed). It also produced a mild

enhancement of SDS foam affinity of acid dyes as evidenced by a slight shift of the liquid fractions toward the center.

Strong effects were observed in 0.01N HCl and 0.01N NaOH. Hydrochloric acid (shown as striped bars in Figure 5) strongly suppressed CPC affinity of acid dyes as did 0.1M NaCl while it also showed a moderate degree of inhibitory action on SDS foam affinity of basic dyes. On the other hand, 0.01N NaOH (shown as checked bars in Figure 5) gave somewhat irregular effects. As expected, it produced significant effects on SDS foam affinity of basic dyes such as neutral red and methyl green, while it gave little effect to some other basic dyes such as Nile blue A and crystal violet. Sodium hydroxide also moderately inhibited CPC foam affinity of DNP amino acids and acid dyes.

Overall results of the above experiments clearly indicate that the positively charged ions are collected by SDS and the negatively charged ions by CPC. Cations such as sodium ions, if added to the surfactant solution, competitively inhibit ionic binding of positively charged samples (such as basic dyes) to the SDS molecule to reduce their foam affinity. Similarly, anions such as chloride ions inhibit CPC foam affinity of negatively charged samples (such as DNP amino acids and acid dyes). Accordingly, sodium chloride, if added in a high concentration, can affect binding of both acidic and basic compounds to the corresponding surfactants. Irregular action of sodium hydroxide to basic dyes, however, remains for further investigation.

The separation of noncolored samples was then observed. The eluates from each collection line were fractionated in separate

Samples		Surfactant SDS	CPC
Nucleotides and Related Compounds	Adenine	L	L
	Adenosine	L	L
	AMP	L	L
	ADP	L	L
	ATP	L	L
	Poly A	L	L
	DNA (Calf Thymus)	L	L
Peptides and Proteins	Gramicidins	L	L
	Mellitin	L	L
	Insulin (Bovine)	F	L
	Hemoglobin (Human)	L	L
	Histone (Calf Thymus)	L	L
	Serum Albumin (Bovine)	L	L
	α Globulin (Human)	L	L
Miscellaneous	Dinitrobenzene	L	L
	Abscisic Acid	L	F
	Indole-3-Acetic Acid	L	F
	Epinephrine	L	L

L: Collected From Liquid Line; F: Collected From Foam Line

Figure 6: Separation of noncolored samples: absorbance measured at 280 nm for each sample. "L" represents eluate observed from liquid collection line and "F" designates eluate from foam outlet. Abscisic acid and indole-3-acetic acid collected with CPC. With SDS as the surfactant, only insulin carried with foam.

test tubes at thirty second intervals and the absorbance of each fraction was measured at 280 nm with a Zeiss spectrophotometer. Figure 6 shows the results of the experiments performed. The letter "L" designates the liquid collection line eluate while the letter "F" represents the eluate from the foam collection line. Among all samples examined, only abscisic acid and indole-3-acetic acid were collected with CPC. Bovine insulin was the only noncolored sample collected with SDS.

The above results clearly indicate that the present method may be effectively utilized for enrichment and separation of these compounds by selecting the proper surfactant. Because of rapid and efficient separation, foam countercurrent chromatography has great potential in the separation and purification of samples in research laboratories.

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