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Foam Separation as a Tool in Chemical Oceanography

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ABSTRACT

A simple batch-type, foam-separation apparatus is described which has produced a 200-fold concentration of added protein from artificial seawater. Essentially total recovery of the protein was achieved from initial concentrations as low as 5 μg/l. The method is rapid and simple in operation and is proposed for use in concentrating surface-active compounds from seawater.

PROBLEM STATUS

This is a final report on one phase of the problem; work on other aspects of the problem continues.

AUTHORIZATION

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FOAM SEPARATION AS A TOOL IN CHEMICAL OCEANOGRAPHY

INTRODUCTION

There is ample evidence to indicate that even in very minute quantities, dissolved organic substances profoundly influence the nature and reactions of both animate and inanimate components of a marine environment (1-5). Most dissolved organic compounds occur in seawater at concentrations in the parts-per-billion range, with the sum of such material rarely exceeding a few parts per million. Total salt concentrations, on the other hand, normally amount to 32 to 37 ppt. A major obstacle to much-needed biochemical research in the oceans is thus the isolation of material sufficiently concentrated to permit subsequent fractionation and characterization of the individual organic compounds.

Ideally, any method for isolating trace organic substances from seawater should satisfy two major criteria: the isolation should be accomplished rapidly, to avoid bacterial modification or nonbiological oxidation of the sample, and in relatively high yield; and the treatment itself should not significantly alter, for identification purposes, the chemical nature of the sample. No such ideal procedure exists. The principal methods that have been used are: solvent extraction (6,7), dialysis (8), adsorption on solid substrates (9,10), and coprecipitation, usually with ferric hydroxide (9,11). Other, less frequently used, methods include: electrodialysis (12), ion exchange (13), and ligand exchange (14). Gas chromatography, preceded by concentration of the compounds in adsorptive cold traps, has been used successfully for separating and measuring minute quantities of light hydrocarbons (15). Most of the methods are either highly specific, or they suffer from low yields, require large solvent volumes, produce large quantities of dilute dialysates, or exesssively alter the compounds on adsorptive surfaces. Many also require inordinately long periods of time for their execution.

It has been known for many years that natural sea foams will concentrate organic matter (16). Furthermore, concentration and separation by foaming has now become a standard method in chemical engineering (17), where it is used, for example, to purify solvents and to recover trace amounts of byproducts from process waste effluents. It has also been used in biochemical research to concentrate and purify enzymes and other proteins (18-20), to separate phospholipids from biological fluids (21), and to purify bile salts (22), to cite only a few examples. It is somewhat surprising, therefore, that serious investigation of the foam separation process as a concentration and preliminary separation technique in chemical oceanography has not been reported. Some recent work has been done with foaming as a means of generating particulate matter from dissolved organic compounds occurring in seawater (23-25), but little or no attention seems to have been directed to the foaming process as a potential tool for studying dissolved organic compounds per se.

The foam separation process becomes more efficient as the solution becomes more dilute (18); thus it works best under the very conditions where many other separation and purification methods become less effective. Furthermore, separations are usually accomplished rapidly and with minimal chemical alteration of labile compounds. Although the process can be used only with surface-active compounds or substances that can be scavenged with naturally occurring or added surfactants, many of the compounds one expects to find in the sea are surface active, and many others would likely be susceptible to scavenging. Proteins, certain polysaccharides, and similar surfactants possessing a

strong affinity for water probably would be concentrated most effectively by foaming, since they are not extractable with nonpolar solvents. Lipoidal and similar extractable substances, particularly the highly surface-active phospholipids, would also be readily concentrated by the foaming technique.

The principle underlying foam separation can be described by the Gibbs adsorption isotherm for an ideal aqueous solution containing a single surface-active solute:

$$\Gamma = -\frac{C}{RT}\frac{d\gamma}{dC}, \tag{1}$$

where

Γ is the surface excess of solute, at equilibrium, in g-mol/cm^2,

C is the concentration of the solute in the sample in g-mol/l,

γ is the surface tension of the solution in dynes/cm,

R is the universal gas constant, 8.314 dyne-cm/mol-°K,

T is the absolute temperature in °K.

Schnepf and Gaden (18) have discussed several limitations on the direct application of the Gibbs equation to real foam systems. Chief among these are: (a) there is no general rule governing the behavior of multicomponent systems, because component interactions may enhance separation in some cases and depress it in others, and (b) Γ is based on the surface area (rather than the volume) of a static surface only under equilibrium conditions. Nevertheless, Eq. (1) is useful for describing the principle involved and for determining whether or not a given solute might be concentrated by foaming.

We are investigating the foam separation process to determine its usefulness for concentrating and isolating organic compounds from seawater. We have chosen protein for our present studies, since it has not, to our knowledge, been reported unequivocally as a natural constituent of seawater. One would expect to find soluble protein in at least trace amounts, however, and its isolation and characterization would be of major significance in marine microbial ecology. This report deals with the quantitative recovery of protein added to an artificial seawater system and describes in detail a simple batch technique we have developed for carrying out foam separations.

THE FOAM TOWER

The basic technique underlying any foam separation is that of producing and collecting large amounts of gas-solution interfaces. This is usually accomplished by allowing bubbles to rise through a column of solution in a "foam tower." Solute-enriched foam is then removed from the top; when it collapses, an enriched solution (the foamate) results. If the surfactant concentration is so low that a stable foam is not produced, the upper part of the solution column nevertheless becomes enriched with the surfactant, provided that turbulent mixing in the tower is not too severe. Collection of this top liquid will give a solution enriched with the solute, although the end product would be more dilute than if it were derived from a stable, well-drained foam. Dorman and Lemlich (26) refer to this procedure as "bubble fractionation" in a "foamless foam column."

Figure 1 is a schematic diagram of the foam separation equipment used in the present work, and Fig. 2 is a photograph of the complete assembly in operation. The tower

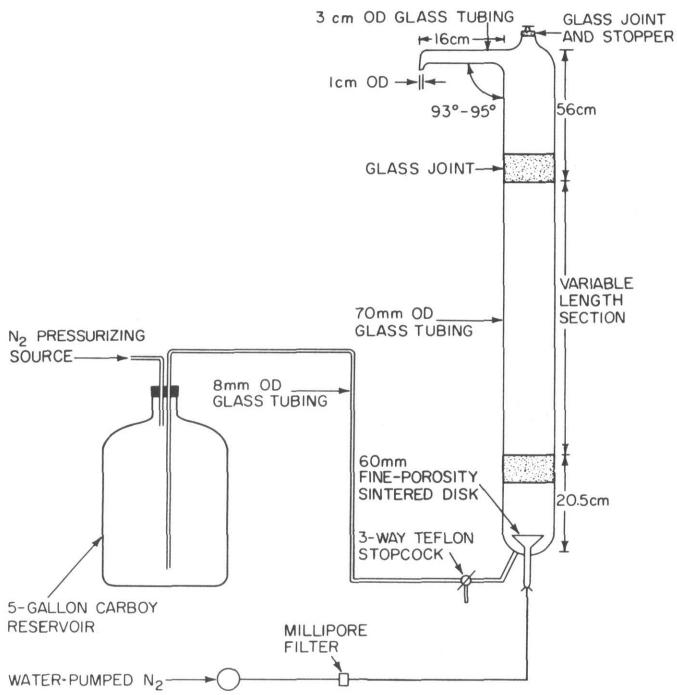


Fig. 1 - Schematic diagram of foam separation apparatus

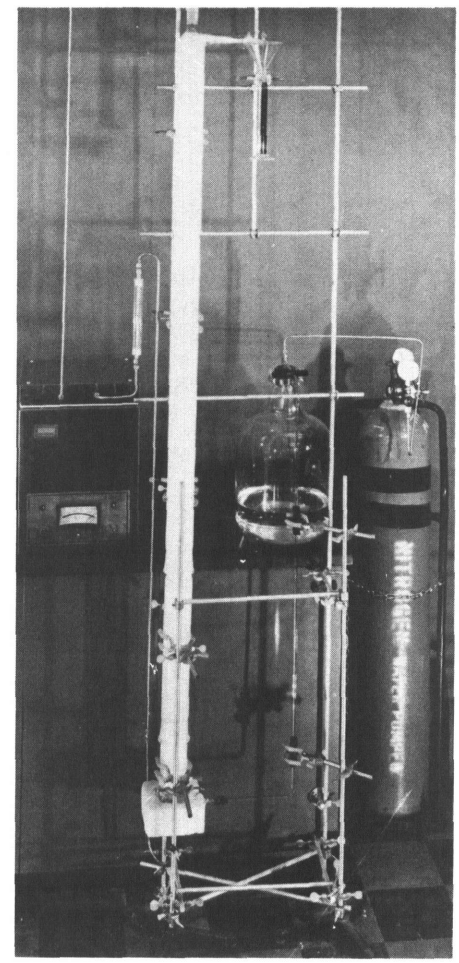


Fig. 2 - Foam separation apparatus in operation. The combustion furnace in the gas input line (left of photo) is not used with high-purity nitrogen.

itself is constructed entirely of Pyrex glass tubing, in three sections. The middle section can be selected from various lengths, providing a capability of foaming different volumes of sample in one run or, if desired, permitting the use of different bubble path lengths. All three sections are made from 70-mm-O.D. tubing to which 71/60 ground-glass joints have been fused.

The top section contains a 29/40 ground-glass joint and stopper at the very top (for access during cleaning) and a sidearm consisting of a 16-cm length of 3-cm Pyrex tubing fused to the wall at an angle of about 5 degrees above the horizontal. The foam flows out through a 10-mm-O.D. vertical tip in this sidearm into an attached collecting vessel, usually a graduated cylinder. There is no foam breaker.

The bottom section contains a 60-mm, fine-porosity (4- to 5.5-μ pores) sintered glass disk (the gas sparger) and a three-way Teflon-bore stopcock attached by means of 8-mm Pyrex tubing. Both the sintered disk and the stopcock are fused to the section, avoiding the use of any cement, stoppers, or O-rings. The Teflon bore of the stopcock is the only point in the entire system where the sample comes in contact with anything other than glass. All ground-glass joints are used ungreased.

The sparging gas, which is usually ultra high-purity, water-pumped nitrogen, passes first through a two-stage regulator and a needle valve. It then flows through a thoroughly washed, cellulose ester membrane, in-line filter (average pore size 0.2 μ), a flow meter, and the sparger.

For prolonged foaming periods, a humidifier is inserted in the line followed by another in-line membrane filter. For periods less than about 3 hr, however, evaporation losses were found to be negligible when 7.5 l or more of sample were being foamed and the sparging gas was nitrogen. The effects of other gases on evaporation were not studied. Our experience to date indicates that combustion of the sparging gas is unnecessary with the ultra high-purity nitrogen, since no organic contamination has been detected. All tubing connections from the gas cylinder to the tower are made of exhaustively cleaned 1/4-in. stainless steel tubing.

The bulk phase (sample) feed system consists of a 5-gallon Pyrex carboy connected to the three-way stopcock by an 8-mm Pyrex tube to which 12/5 ground-glass ball-and-socket joints have been fused. The sample is transferred by pressurizing the carboy with nitrogen.

EXPERIMENTAL PROCEDURE

Our procedure for carrying out foam separations with this equipment will be described in three parts: (a) preparation of the apparatus and sample, (b) the foaming operation, (c) analytical procedures. It must be emphasized that we are using foam separation as a microchemical technique; i.e., we are attempting to work with solutions containing solutes in the parts-per-billion range. Consequently, the utmost care is taken to minimize both contamination and loss of material.

Preparation of Apparatus and Sample

All glassware is thoroughly cleaned in acid dichromate solution, rinsed directly with ordinary distilled water, and finally rinsed in water (designated "3-D water") that has been prepared by passing tap water through deionizing and organic-removal resin beds, followed by distillation from acid permanganate and then alkaline permanganate solutions. The freshly cleaned glassware is immediately protected from atmospheric contamination by closing it off with aluminum foil. Cleaning is accomplished immediately prior to use whenever possible.

The stainless steel tubing used for gas delivery is first flushed with a detergent solution, rinsed thoroughly with distilled water, and finally rinsed with acetone. This treatment is followed by a thorough purging with ultra high-purity nitrogen. Subsequent cleaning is considered necessary only whenever the system has suffered prolonged exposure to atmospheric contamination.

At first, artificial seawater (ASW) was prepared by the procedure of Kester, Duedall, Connors, and Pytkowicz (27), except that NaF was omitted, the $MgCl_2 \cdot 6H_2O$, $SrCl_2 \cdot 6H_2O$, and $CaCl_2$ were not added volumetrically, and the salts were not oven dried before being weighed. The 3-D water was used throughout. This ASW was then "prefoamed" in the tower, and approximately 100 ml of wet, unstable foam collected and discarded. The residual fraction was withdrawn from the tower and used to prepare the protein solution. This procedure appeared to remove all detectable surface-active contaminants and large amounts of iron-bearing precipitates, presumably ferric hydroxide and phosphate. We have designated this procedure "Method A" and the product "ASW_a."

In Method A, it was observed that substantial numbers of small particles remained in the residual phase after prefoaming, although large amounts of particulate matter also had been floated over in the foam and discarded. We considered it possible that these particles were adsorbing some protein and causing losses on any that did not float over during the subsequent separation run. Since it was likely that many of these particles

were formed *de novo* from surface-active contaminants during the prefoaming run (see the discussion section), we hoped to eliminate the need for prefoaming the ASW by separately purifying those reagents that seemed to contain the contaminating surfactants: viz $CaCl_2$ and $MgCl_2 \cdot 6H_2O$.

All salts, with the exception of NaCl and Na_2SO_4, were now added from stock solutions. The concentrated $CaCl_2$ solution was heavily contaminated with colored insoluble material when first prepared from reagent grade salt and so was filtered through an exhaustively washed cellulose membrane filter. It was not necessary to filter the $MgCl_2 \cdot 6H_2O$ solution. Each of these stock solutions was then foamed in a small-capacity tower, and semistable, wet foam (from $CaCl_2$) or stable, brownish froth (from $MgCl_2$) was collected and discarded until no further traces were seen. The residual solutions were then withdrawn, and the $MgCl_2$ solution was freed of particles generated during foaming by filtering it through a coarse-porosity sintered glass funnel. Both solutions were standardized by chelometric titration of their cations.

In preparing the ASW, appropriate volumes of the Ca, Mg, and Sr stock solutions were combined and diluted in one vessel, and the remaining salts were combined and diluted in a second vessel in the manner of Kester, Duedall, Connors, and Pytkowicz (27). The two diluted solutions were then combined, and the whole was made up to volume (usually 15.63 l) with 3-D water. The final salinity was 35 ‰. This method of preparation is designated "Method B" and the product "ASW_b."

An appropriate volume of ASW, prepared by Method A or B, as desired, was then placed in the 5-gallon carboy to be used as the sample feed reservoir. A stock solution containing a known concentration of crystalline bovine serum albumin (BSA) in 3-D water was freshly prepared for each run. This was diluted appropriately, and the desired volume was added to the reservoir with gentle swirling to minimize particle formation through foaming (see the discussion section). The protein was added only moments before introducing the sample into the tower. Portions of the stock solution were also used to prepare the analytical standards.

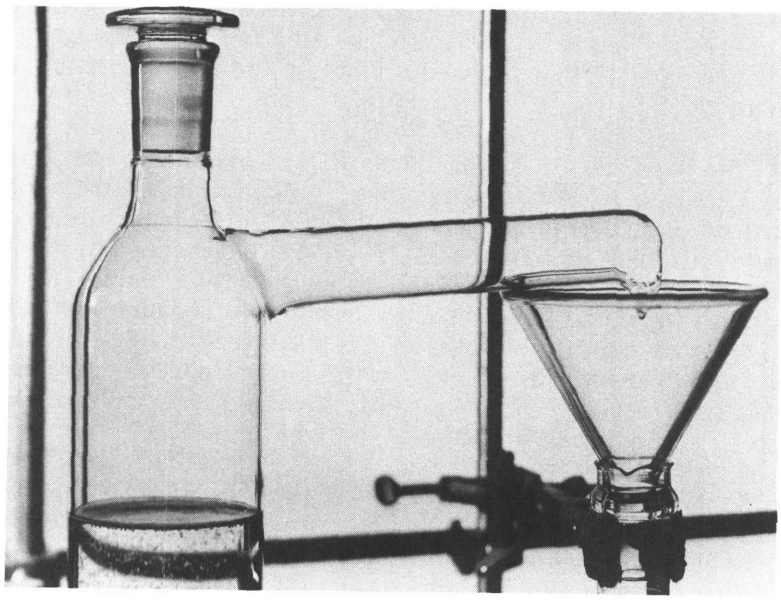
The Foaming Operation

The sample was introduced into the tower by opening the three-way stopcock and pressurizing the reservoir to about 2 psi. The gas flow to the sparger was regulated so that the pressure at the sintered disk constantly balanced the hydrostatic pressure as the sample level rose. This is necessary to prevent backflow of sample into the gas delivery system and also to prevent premature enrichment of the sample surface. Particles which would be formed during premature foaming can be lost by attachment to the walls and especially the joints during filling.* Consequently, the gas flow rate was not adjusted to the desired level until the top of the liquid was above the uppermost joint.

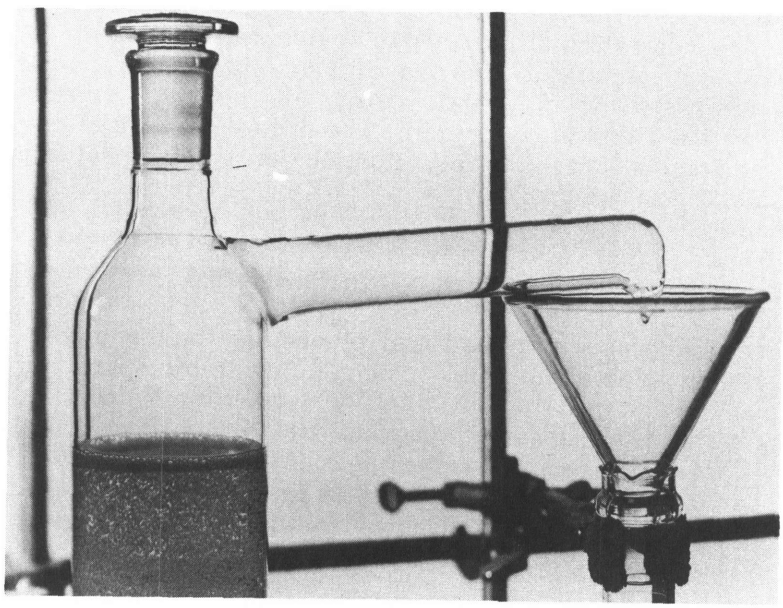
When the liquid surface has risen above the top joint, the gas flow was established at the desired rate and, after expansion of the liquid, column filling was stopped when the surface was just below the delivery arm. Time was then allowed for the foam, if any was produced, to rise to maximum height. This always occurred within 30 to 120 sec of starting the gas flow (Figs. 3a to 3d).† Foam was collected before any significant amount

*Early experience with hemoglobin showed that at least with highly surface-active proteins, serious losses occurred on the walls of the tower during foaming. We were unable to obtain consistent recovery values with hemoglobin for that reason.

†Figures 3a to 3d are of foams produced from natural samples of neritic seawater. The appearance is very similar, however, to what was seen with BSA in ASW.

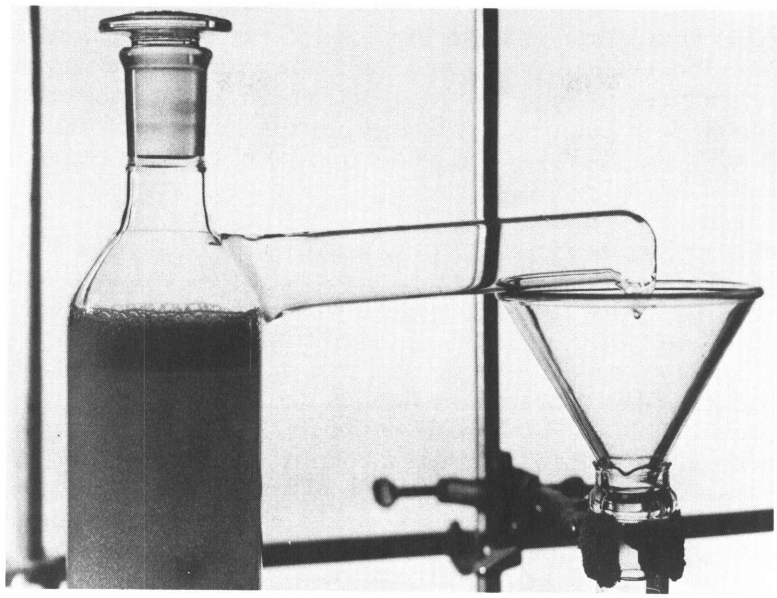


(a) 20 sec

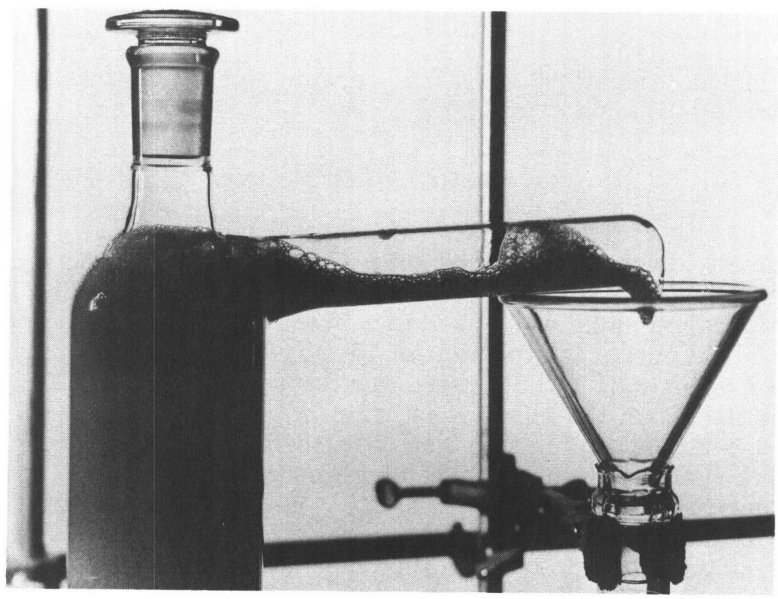


(b) 25 sec

Fig. 3 - Foam formation at the top of the liquid column at various times after the initiation of sparging. Natural seawater sample.



(c) 35 sec



(d) 50 sec

Fig. 3 - Foam formation at the top of the liquid column at various times after the initiation of sparging. Natural seawater sample (Continued).

of refluxing could occur (see the discussion section). This was done by adding, when necessary, a few milliliters of sample via the stopcock to raise the liquid-foam interface into the arm.* The foam thereupon expands into the arm, where it drains and eventually is pushed over into the receiver by the escaping gas (Fig. 3d) or, if necessary, by further small additions of sample. If no foam was produced, small volumes of top liquid were collected immediately after the operator detected evidence of enrichment at the top of the liquid column or, in any event, within 2 min.

With the artificial samples used in this work, virtually all of any foam that could be produced was collected at a single gas flow rate. Traces of wet, unstable foam often remained, however. Early in the work, our procedure was next to raise the gas flow to a higher rate, allow the system to aerate for a period of time, and periodically force small quantities of top liquid and any traces of foam over into the receiver. When this should be done is largely a matter of the operator's judgment and experience, derived chiefly from observing individual bubbles and the mass of breaking bubbles for signs of stability. Care must be taken to avoid collecting too great a volume, or the resulting foamate may be too dilute for analysis. It was necessary to drain 50 to 150 ml of liquid from the tower via the three-way stopcock to allow for expansion of the liquid column when the gas flow was increased. Final positioning of the interface was done by running in small amounts of sample, as before. Four gas flow rates were routinely used in succession, viz 550, 750, 875, and 1000 ml/min (Method I). Later, however, we used only a single flow rate of 1000 ml/min (Method II). The reasons for using four flow rates in succession are given in the discussion section. The procedure followed in going to the next higher flow rate was the same for each rate change. Our normal procedure was to aerate at each flow rate for a period of 30 min. All runs were made at room temperature (21 to 23°C).

The separation run was terminated when the operator considered that no further evidence of foam or enriched top liquid existed.

Analytical Procedures

Protein was ordinarily determined by the Folin-Ciocalteau phenol method using a modification of the procedure of Lowry, Rosebrough, Farr, and Randall (28). Cold 50-percent aqueous trichloroacetic acid was added to a final concentration of 8 percent to replicate aliquots (usually 5 ml) of the foamate in 10-ml centrifuge tubes. The tubes were allowed to stand overnight at 3°C and were then centrifuged under refrigeration. The supernatants were decanted, the tubes were drained, and the precipitates were dissolved in 0.5 ml of 1.0-N NaOH. Color was then developed in the original centrifuge tubes, using the reagent concentrations specified by Bennett (29). Absorption was measured at 660 nm in a Spectronic-20 colorimeter using 1-in. cuvettes. Reagent blanks and standards were run with the samples through the entire procedure. Standards were prepared in ASW by diluting the stock protein solution used in the separation run to a concentration near that expected in the foamate, assuming 100-percent recovery. Good precision was obtained down to protein levels of 2 μg/ml of foamate.

When protein concentrations in the foamate were less than about 2 μg/ml, a modification of the membrane-binding procedure of Kihara and Kuno (30) was employed in conjunction with the phenol method (as described above) to estimate the total protein. Since the procedure of Kihara and Kuno is applicable only to dissolved protein, and substantial

*Addition of 20 ml (a common requirement) of sample containing 25 μg/l of BSA represents an addition of 0.5 μg of surfactant into a volume of e.g. 7.5 l. This is considered insignificant in these experiments.

numbers of protein particles were formed during foaming (see the discussion section), preliminary fractionation of the foamate was effected by centrifugation. The supernatant was then analyzed by the modified Kihara and Kuno procedure, and the sediment was analyzed by the phenol method, since the protein concentration in the sediment fraction was always much higher.

In our modification of the Kihara and Kuno procedure, the supernatant was slowly passed through a well-washed cellulose membrane filter (average pore size 0.2 μ) under very slight vacuum. The filter was then treated according to Kihara and Kuno, except that 15 μg of amido black (in 3 ml) was used, and the protein was bound directly from ASW solution. A calibration curve was prepared from standard solutions of BSA in ASW, and a linear relationship between protein concentration and the amount of dye bound was demonstrated in the range used; viz 5 to 50 μg of total protein.

RESULTS

Our work thus far has been exclusively with batch separations. More elaborate processes, involving recycling, continuous-feed, and multistage operations, will undoubtedly prove useful for many purposes and will be investigated in due course. We believe, however, that the single-stage batch operation will have wider application in marine science because of its extremely simple apparatus and operating requirements.

Table 1 shows the results obtained with BSA dissolved in ASW at various concentrations. It is apparent that recoveries near 100 percent were regularly obtained, even when the initial concentration of protein was as low as 5 μg/l. Furthermore, it appears that the extent of recovery was independent of initial concentration, the total weight of protein foamed, and the volume of foamate collected, within the range of values studied.

The volume of foamate collected is, of course, critical in determining the concentration of surfactant available for analysis. The limiting factor in this method at extreme sample dilutions is probably our ability to analyze the resulting foamate. It can be seen from the table that, as more experience was obtained with the procedure, it became possible to collect smaller volumes of foamate without sacrificing the quantitative aspects of the recovery. This is reflected in the higher concentration factors that we achieved in the later stages of the work.

The two low recoveries (61 and 68 percent) at the 5-ppb level were obtained early in the work and probably resulted from using the Kihara and Kuno method of analysis without preliminary fractionation of the foamate into particulate and dissolved phases. This would have resulted in low analytical results, since one would not expect all of the protein contained in the interior of particles to participate in dye binding. Later 5 μg/l runs showed higher recoveries.

The runs in which the ASW was prepared by Method A (ASW prefoamed) gave slightly lower recoveries than comparable runs in which the ASW was prepared by Method B (ASW not prefoamed; $MgCl_2$ and $CaCl_2$ prepurified). The Method A runs were made early in the experimental series and were not repeated. The reasons for the differences cannot be explained at this time.

DISCUSSION

Excellent discussions of both the theory and practice of foam separation have been published by Rubin and Gaden (17), Bikerman (31), Wace and Banfield (32), and Haas (33). A recent review and extensive discussion of the technique has been published by Lemlich

Table 1
Results of Foam Separation Runs with Bovine Serum Albumin Dissolved in Artificial Seawater

Date	Initial BSA Conc'n (μg/l)	ASW Prep'n Method	Foaming Method	Volume Foamed* (l)	Volume Foamate (ml)	Amount BSA Orig (μg)	Amount BSA in Foamate (μg)	Percent Recovery	BSA Lost (μg)	BSA Conc'n in Foamate (μg/ml)	Concentration Factor†
11/2/67	150	A	I	7.58	92	1137	1050	92	74	11.4	76
12/21/67	150	A	I	7.76	96	1163	1086	93	77	11.3	75
5/8/68	75	A	I	7.65	62	574	506	88	68	8.2	109
5/23/68	75	A	I	7.69	70	577	510	88	67	7.3	97
8/2/68	75	B	I	13.94‡	137	1069	1058	99	11	7.7	103
8/5/68	75	B	I	7.75	76	581	555	96	26	7.3	97
2/19/69	75	B	II	7.31	65	548	560	102	—	8.6	115
2/19/69	75	B	II	7.33	76	549	558	102	—	7.6	101
8/20/68	25	B	I	7.69	52	192	182	95	10	3.5	140
12/21/68	25	B	I	7.62	63	191	193	101	—	3.1	124
2/5/69	25	B	II	7.29	31	182	187	103	—	6.0	240
2/11/69	25	B	II	7.30	33	182	186	102	—	5.6	224
2/12/69	25	B	II	7.25	31	181	183	101	—	5.8	232
2/12/69	25	B	II	7.25	34	181	194	107	—	5.7	228
3/6/69	15	B	II	7.28	27	109	110	101	—	4.1	273
3/6/69	15	B	II	7.28	43	109	107	98	—	2.5	167
8/28/68	5	B	I	7.67	94	38	24	61	14	0.26	52
9/5/68	5	B	I	7.66	105	38	26	68	12	0.25	50
3/18/69	5	B	II	7.27	31	36	31	86	5	1.0	200
3/18/69	5	B	II	7.27	38	36	33	92	3	0.9	180

*The actual volume foamed depended upon how much additional sample was run in to force over the foam and/or top liquid.
†Concentration in foamate/concentration in original sample.
‡This run was made with a tower fabricated with a longer midsection to give a capacity of about 14 l.

(34). Our discussion, therefore, will cover only those factors which were of major significance in the development of our particular apparatus and technique or which may require special consideration in applications to marine samples.

Two potentially rate-limiting processes occur in foam separations: transfer of the surfactant from the dissolved to the adsorbed state (mass transfer) and transport of the adsorbed surfactant to the top of the liquid column (solute throughput). The rate of mass transfer depends primarily on the diffusion coefficient of the solute molecule, the degree of turbulence in the liquid column, and the amount of surface area per unit volume to which the surfactant molecules can migrate. Solute throughput, on the other hand, is the quantity of adsorbed surfactant transported per unit cross-sectional area of liquid column per unit time. Both processes depend strongly on bubble diameter and gas flow rate (32). Bubble diameter, in turn, depends on the porosity of the gas sparger, the pressure drop across the sparger, and certain physical properties of the solution, chiefly surface tension, viscosity, and density. The principal variable which influenced bubble diameter in this work was the pressure drop across the sparger. The pressure drop is a function of the gas flow rate. Thus, gas flow rate assumes a position of major importance in foam separation operations.

Wace and Banfield (32) have shown that for a tower to produce maximum solute throughput with bubbles near the optimum diameter, it should have a gas flow rate of 1.8 cm/sec per square centimeter of liquid column cross-sectional area. They have further determined that a bubble diameter of 0.8 mm is optimum, and that deviations of an order of magnitude from this figure reduce the throughput only by a factor of two. The gas flow rate of 1.8 cm/sec per square centimeter cross section with a bubble diameter of 0.8 mm is the flooding velocity: i.e., the flow rate at which the liquid-foam interface disappears.

While these conditions will, according to Wace and Banfield, produce maximum solute throughput, they would not be expected to produce an optimum separation in our practical sense. The high-density foam resulting from insufficient drainage would produce a highly dilute foamate. The considerable turbulence in the enriched zone at the top of the liquid column probably would drive some adsorbed material back down into the column.* Thus, some gas flow rate below the flooding velocity would likely be optimum in the sense of producing a rapid buildup of a foam that would become well drained and yield a concentrated foamate.

The initial decision to use four gas flow rates in succession was an empirical attempt to generate nearly optimum conditions. It was thought that the lower initial flow rate would provide the best conditions for producing a distinct, well-drained foam by keeping turbulence in the foam and at the foam-liquid interface to a minimum. It was also thought that by increasing the flow rate as the sample concentration of surfactant was decreased by removal, more surface area would be provided per unit volume per unit time. Although time is not highly important in a batch process, it was felt that the rapid production and collection of foam was nevertheless desirable, for reasons to be explained below in connection with micelle formation. Finally, it was felt that by using a succession of flow rates, at least one would produce a nearly optimum bubble size.

Subsequently we found that the range of flow rates used did not materially alter the bubble diameter. The bubbles were observed to have diameters between 0.1 and 2 mm; thus, our tower was operating under nearly optimum conditions with respect to bubble diameter. At an inlet flow rate of 1000 ml/min (the maximum used), the flow rate is

*This latter would be particularly detrimental in the case where no foam was produced, as in the "bubble separation" of Dorman and Lemlich (26), cited earlier.

0.46 cm/sec per unit cross section of tower. This is far below the value prescribed by Wace and Banfield (32) for maximum throughput. However, since essentially complete recoveries were obtained at all surfactant concentrations tested, it would appear that the lower rates were entirely adequate for our purpose, although it cannot be said whether they were optimum in any theoretical sense. Furthermore, it is apparent from Table 1 that complete recoveries were as readily obtained by Method II (1000 ml/min only) as by Method I (four rates in succession). A detailed study of bubble diameter and gas flow rate parameters has not yet been made with our system.

All of our foamates, from both natural and artificial marine samples, either have been visibly turbid or have shown significant light scattering with a Tyndall beam. This has raised questions concerning the significance of particle production and froth flotation processes in the foam separation method. "Froth flotation" is a term applied to the process of mechanically floating off particulate matter by aerating a solution to which surfactants have been added to produce foam and to form collecting surfaces. Since froth flotation involves a three-phase system, it is fundamentally different from foam separation, which involves adsorption of dissolved material from a homogeneous liquid phase. Superficially, however, the two processes are very similar and often coexist. The classical use of froth flotation has been in the "beneficiation" of ores (31), but it has also been applied to the removal of microorganisms and solids from many types of systems. A related process, termed "ion flotation," involves the collection of weakly surfactant or nonsurfactant ionic compounds or metal ions by complexing them with an appropriate surface-active scavenger and foaming the solution (35). In many cases, the complex is insoluble, so that true froth flotation occurs.

The observations with hemoglobin (mentioned above) indicated that at least some of it was converted to particulate form. Presumably, this conversion occurred either through adsorption onto the surfaces of preexisting particles or *de novo* through compression, fragmentation, and aggregation of insoluble monolayers as postulated by Sebba (35). Similar mechanisms have been suggested by other investigators (23,24) as a basis for *de novo* particle production in the sea.

We found that in two 5 μg/l runs on March 18, 1969, essentially all of the 36 μg of BSA introduced into the tower in each run was recovered as particulate (sediment) protein after lightly centrifuging the foamate (at 2440 g). A small amount of color was bound from the supernatant phase in the Kihara and Kuno procedure. This was interpreted as amounting to about 1.5 μg of protein in each case, but the amount of color eluted was well below the reliable limit (5 μg) for the procedure.

Experiments were next conducted in which BSA at a final concentration of 25 μg/l was added to ASW_b which had been ultrafiltered to remove all particles. The resulting foamates were visibly turbid, and microscopic examination showed many particles having the appearance of typical "aggregates" reported for natural samples (24,25). This latter work provides strong evidence for the production *de novo* of particles during a foam run. The ultrafiltration appears to rule out a need for preexisting particulate matter, although it does not rule out the possibility that adsorption may have taken place on mineral particles formed in the liquid column during the foam run. Studies are now in progress with the electron microscope to determine whether mineral nuclei are involved in the production of these particles.

In any event, it appears that particle production and froth flotation are important factors in this type of foam separation. It is surprising that more mention of particle production during foaming has not been made in the foam separation literature. Bader and Shütz (22), however, did note that crystals of both cholic acid and mixed fatty acids were formed in their foams of impure bile salts, and Schütz (36) reported that particulate matter occurred in nearly all his foamates. Karger (37) noted the formation of an

insoluble material while foaming a mixture of sodium sulfate and 1-naphthylamine, but he did not characterize it. Schütz (36) noted that the particles obtained when foaming proteins were a consequence of surface denaturation. Thuman, Brown, and McBain (38) noted the formation of an insoluble residue when foaming a protein solution under maximum foam-producing conditions. The residue was not seen when weak foams were produced, and its formation was also attributed to surface denaturation. However, it follows from the work described here above that *de novo* particle production can occur in the total absence of a stable foam, inasmuch as no stable foams were produced when the initial BSA concentration was 25 μg/l or less.

When a surfactant's concentration reaches a unique level, the critical micelle concentration (CMC), there is an increased tendency for the molecules to assume a thermodynamically more stable configuration by arranging themselves into micelles. A number of surfactant molecules come together and assume a configuration in which their hydrophobic ends together form a relatively hydrophobic internal environment while their hydrophilic ends remain in the polar exterior. Micelle formation may be attended by a loss or diminution of surface activity (35), so it can be considered as a possibly detrimental process in foam separations. The CMC is usually lowered by the presence of salts (35) and is probably often exceeded in the enriched top liquid of a foam or bubble separation tower. The molecules, once formed into micelles, can be transported down into the liquid column by foam drainage or turbulence, and it is thought (35) that they may be slow to dissociate back into the nonaggregated, more active state.

Definitive experiments to assess the importance of micelle formation in our work have not been conducted. A simple experiment was carried out, however, in which foamate collection was delayed 15 and 30 min after the initiation of foaming, permitting a substantial amount of internal refluxing to occur. Subsequent analyses showed a 7- and 15-percent decrease in recovery, respectively, when compared with immediate collection. This experiment was performed with ultrafiltered ASW_b, and repetition with water that had not been ultrafiltered showed no significant decrease in recovery with refluxing up to 90 min. This leads to speculation that the particles known to be present in ASW_b may have adsorbed some of the surfactant and thereby reduced the tendency to form micelles. This in turn raises a host of new questions concerning the role of particulate matter in surfactant recovery which eventually will have to be examined. However, in the absence of definitive information on the role of micelle formation in degrading foam separations, we decided to collect the surfactant as soon as possible after it was brought to the top of the liquid column.

The foam tower should be designed in accordance with certain basic criteria. Turbulence within the liquid column must be kept to a minimum, especially near the liquid-foam interface, for reasons already discussed. By keeping the diameter of the tower close to that of the glass frit used as the sparger, channeling was reduced. The area of maximum turbulence in the present apparatus was found to occur in the vicinity of the joints, where there was a slight constriction (Fig. 4). Consequently, it was necessary to avoid placing joints close to the top of the tower. A distance of about 60 to 70 cm was sufficient in the present tower to allow most of the joint-induced turbulence to dissipate before it could affect the top of the liquid column.

It is equally important that the design facilitate rapid and discrete collection of the foamate. Where stable foams are produced, the region in which the foam will concentrate should allow expansion to facilitate drainage. At the same time, the design must permit the operator to collect the foam without diluting it with large amounts of the liquid phase. The slightly inclined arm proposed by Haas (33) was selected, as it satisfies these conditions. The foam expands into the arm, becomes well drained in the absence of a vertical gas flow, and is easily collected, with a minimum carryover of liquid (Fig. 3d). This design also permits good control when collecting enriched top liquid in cases

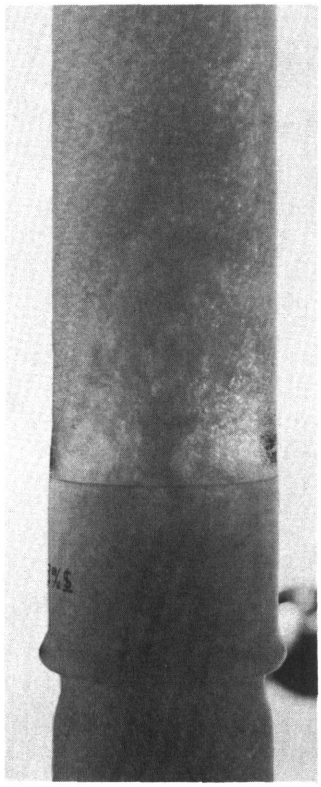


Fig. 4 - Example of turbulence occurring in the vicinity of a joint

where a stable foam is not produced. While the concentration of protein in the "foamates" obtained from runs where no stable foams were produced was less than that from runs with higher initial concentrations, the concentration factors were among the highest obtained.

The advantages of the foam separation technique described in this report lie in its simplicity, rapidity, and high degree of efficiency (insofar as it has been evaluated). Large volumes of seawater can be processed in minutes. The physical and chemical environment remains virtually unchanged, when compared with most other methods, and biological modifications of the material are minimized because of the rapidity of the separation. External contamination introduced through excessive sample handling or the addition of "reagent grade" chemicals is also minimized.

Our previous work with foaming natural seawater and pure phytoplankton cultures has resulted in the isolation of mixtures which we believe — on the basis of solvent affinity, chromatographic behavior, and functional group tests — to have contained proteins and/or humic acids, fatty acids, polysaccharides, and possibly phospholipids and steroids. However, these foams were always heavily contaminated with detergentlike substances eluted from the membrane filters used to remove cells and detritus, and this hampered the analysis. Moreover, all types of particle-separation procedures we have tried since have significantly modified the samples in some manner. Since particle removal is the only preliminary treatment required of the sample, removal so as to avoid this contamination with the products of cell rupture or with organic matter not truly dissolved constitutes the major problem to be solved.

The major disadvantages of the method are that it is specific for surface-active compounds and substances that can be scavenged by surfactants, and the native state of macromolecules may be altered. In general, the latter will not interfere with chemical identification, but may seriously hamper determination of the native-state configuration. In any event, foam separation appears to offer a method for rapidly and easily concentrating an important fraction of the dissolved organic matter in the ocean.

REFERENCES

1. Johnston, R., "Biologically Active Compounds in the Sea," J. Mar. Biol. Assn. U.K. 34:185-195 (1955)

2. Lucas, C.E., "External Metabolites in the Sea," Deep-Sea Research 3 (Suppl.):139-148 (1961)

3. Khaylov, K.M., "Dynamic Marine Biochemistry—Development Prospects," Oceanology (Transl.) 5:1-9 (1965)

4. Chave, K.E., "Carbonates: Association with Organic Matter in Surface Seawater," Science 148:1723-1724 (1965)

5. Bader, R.G., Hood, D.W., and Smith, J.B., "Recovery of Dissolved Organic Matter in Sea-Water and Organic Sorption by Particulate Material," Geochim. Cosmochim. Acta 19:236-243 (1960)

6. Slowey, J.F., Jeffrey, L.M., and Hood, D.W., "The Fatty-acid Content of Ocean Water," Geochim. Cosmochim. Acta 26:607-616 (1962)

7. Ushakov, A.N., Vityuk, D.M., Vaver, V.A., and Bergel'son, L.D., "Fatty Acids in Black Sea Water," Oceanology (Transl.) 6:726-728 (1966)

8. Ogura, N., "Method of Concentration of Dissolved Organic Substances from Seawater by Dialysis," J. Oceanogr. Soc. Japan 21:206-211 (1965)

9. Williams, P.M., and Zirino, A., "Scavenging of 'Dissolved' Organic Matter from Sea-Water with Hydrated Metal Oxides," Nature 204:462-464 (1964)

10. Schaefer, H., "Isolierung von gelosten Kohlenhydraten aus dem Meerwasser," Helgolander Wiss. Meeresuntersuch. 12:253-259 (1965)

11. Chapman, G., and Rae, A.C., "Isolation of Organic Solutes from Sea Water by Co-precipitation," Nature 214:627-628 (1967)

12. Jeffrey, L.M., and Hood, D.W., "Organic Matter in Sea Water; An Evaluation of Various Methods for Isolation," J. Marine Research 17:247-271 (1958)

13. Schaefer, H., "Isolierung von gelosten organischen Verbindungen aus dem Meerwasser unter besonderer Berucksichtigung der Aminosauren," Helgolander Wiss. Meeresuntersuch. 12:239-252 (1965)

14. Siegel, A., "A New Approach to the Concentration of Trace Organics in Seawater," in Olson, T.A., and Burgess, F.J., editors, "Pollution and Marine Ecology," 1967, pp. 235-256

15. Swinnerton, J.W., and Linnenbom, V.J., "Gaseous Hydrocarbons in Sea Water: Determination," Science 156:1119-1120 (1967)

16. Skopincev, N.A., "Organic Matter in Sea Water and Foam in the South-eastern Part of the Caspian Sea (Oct.-Nov. 1936)," Compt. Rend. Doklady Akademii Nauk SSSR 18:453-458 (1938)

17. Rubin, E., and Gaden, E.L., Jr., "Foam Separation," Chap. 5 in "New Chemical Engineering Separation Techniques," Schoen, H.M., editor, 1962

18. Schnepf, R.W., and Gaden, E.L., Jr., "Foam Fractionation of Proteins: Concentration of Aqueous Solutions of Bovine Serum Albumin," J. Biochem. Microbiol. Eng. 1:1-11 (1959)

19. Charm, S.E., Morningstar, J., Matteo, C.C., and Paltiel, B., "The Separation and Purification of Enzymes Through Foaming," Anal. Biochem. 15:498-508 (1966)

20. Bader, R., Schütz, F., and Stacey, M., "A Crystalline Serum Mucoprotein with High Choline-Esterase Activity," Nature 154:183-184 (1944)

21. Enhörning, G., Fujiwara, T., and Adams, F.H., "Surface-Active Material in Fetal Lung Fluid Concentrated by Foaming," Tohoku J. Exp. Med. 84:250-255 (1964)

22. Bader, R., and Schütz, F., "Fractionation by Adsorption and Crystallization on Foam. Part II. Experiments with Bile Salts," Trans. Faraday Soc. 42:571-576 (1946)

23. Sutcliffe, W.H., Jr., Baylor, E.R., and Menzel, D.W., "Sea Surface Chemistry and Langmuir Circulation," Deep-Sea Research 10:233-243 (1963)

24. Riley, G.A., "Organic Aggregates in Seawater and the Dynamics of their Formation and Utilization," Limnol. Oceanogr. 8:372-381 (1963)

25. Wilson, D.F., and Hiller, A.J., "Organic Particulates and Dissolved Substances," (Secret Report, Unclassified Title) Proc. 4th Tech. Rev. Surface Effects Program, ONR-19, p. 252 (1964)

26. Dorman, D.C., and Lemlich, R., "Separation of Liquid Mixtures by Non-Foaming Bubble Fractionation," Nature 207:145-146 (1965)

27. Kester, D.R., Duedall, I.W., Connors, D.N., and Pytkowicz, R.M., "Preparation of Artificial Sea Water," Limnol. Oceanogr. 12:176-179 (1967)

28. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., "Protein Measurement with the Folin Phenol Reagent," J. Biol. Chem. 193:265-275 (1951)

29. Bennett, T.P., "Membrane Filtration for Determining Protein in the Presence of Interfering Substances," Nature 213:1131-1132 (1967)

30. Kihara, H.K., and Kuno, H., "Microassay of Protein with Micro Cellulose Membrane Filters," Anal. Biochem. 24:96-105 (1968)

31. Bikerman, J.J., with Perri, J.M., Booth, R.B., and Currie, C.C., "Foams: Theory and Industrial Applications," New York:Reinhold, 1953

32. Wace, P.F., and Banfield, D.L., "Foam Separation," U. K. Atomic Energy Research Establishment, AERE-R5189 (1966)

33. Haas, P.A., "Engineering Development of a Foam Column for Counter-current Surface-Liquid Extraction of Surface-Active Solutes," Oak Ridge National Laboratory, ORNL-3527 (1965)

34. Lemlich, R., "Adsorptive Bubble Separation Methods," Ind. Eng. Chem. 60(10):16-29 (1968)

35. Sebba, F., "Ion Flotation," Amsterdam:Elsevier, 1962

36. Schütz, F., "Fractionation by Adsorption and Crystallization on Foam. Part I. Method and Principles," Trans. Faraday Soc. 42:437-444 (1946)

37. Karger, B.L., "Studies on Foam Fractionation and Application to Lipoid Materials," U.S. Army Natick Labs., Tech. Report 66-25-FD (1966)

38. Thuman, W.C., Brown, A.G., and McBain, J.W., "Studies of Protein Foams Obtained by Bubbling," J. Am. Chem. Soc. 71:3129-3135 (1949)

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13. ABSTRACT

A simple batch-type, foam-separation apparatus is described which has produced a 200-fold concentration of added protein from artificial seawater. Essentially total recovery of the protein was achieved from initial concentrations as low as 5 μg/l. The method is rapid and simple in operation and is proposed for use in concentrating surface-active compounds from seawater.

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