Techniques for Extraction of Foraminifers and Ostracodes from Sediment Samples

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Regional and headquarters establishments of Ocean Science and Surveys ceased publication of their various report series as of December 1981. A complete listing of these publications and the last number issued under each title are published in the *Canadian Journal of Fisheries and Aquatic Sciences*, Volume 38: Index to Publications 1981. The current series began with Report Number 1 in January 1982.

Rapport technique canadien sur l'hydrographie et les sciences océaniques

Ces rapports contiennent des renseignements scientifiques et techniques qui constituent une contribution aux connaissances actuelles mais que l'on ne trouve pas normalement dans les revues scientifiques. Le sujet est généralement rattaché aux programmes et intérêts du service des Sciences et Levés océaniques (SLO) du ministère des Pêches et des Océans.

Les rapports techniques peuvent être considérés comme des publications à part entière. Le titre exact figure au-dessus du résumé du chaque rapport. Les résumés des rapports seront publiés dans la revue Résumés des sciences aquatiques et halieutiques et les titres figureront dans l'index annuel des publications scientifiques et techniques du Ministère.

Les rapports techniques sont produits à l'échelon régional mais sont numérotés et placés dans l'index à l'échelon national. Les demandes de rapports seront satisfaites par l'établissement auteur dont le nom figure sur la couverture et la page de titre. Les rapports épuisés seront fournis contre rétribution par des agents commerciaux.

Les établissements des Sciences et Levés océaniques dans les régions et à l'administration centrale ont cessé de publier leurs diverses séries de rapports depuis décembre 1981. Vous trouverez dans l'index des publications du volume 38 du *Journal canadien des sciences halieutiques et aquatiques*, la liste de ces publications ainsi que le dernier numéro paru dans chaque catégorie. La nouvelle série a commencé avec la publication du Rapport n° 1 en janvier 1982.

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ABSTRACT

Thomas, F.C. and Murney, M.G. 1985. Techniques for extraction of foraminifers and ostracodes from sediment samples. Can. Tech. Rep. Hydrogr. Ocean Sci. 54: vi + 24 p.

Several methods of disaggregating sediment samples for analysis of shelly microfossils, such as foraminifers and ostracodes, are regularly used at the Atlantic Geoscience Centre, Bedford Institute of Oceanography, Dartmouth, N.S. This paper describes and illustrates these methods, with a view towards instructing the inexperienced student in their uses. Also included are a brief source list for materials, and a selected bibliography of publications dealing with these and other techniques.

RÉSUMÉ

Thomas, F.C. and Murney, M.G. 1985. Techniques for extraction of foraminifers and ostracodes from sediment samples. Can. Tech. Rep. Hydrogr. Ocean Sci. 54: vi + 24 p.

A l'Institut Océanographique de Bedford, Dartmouth, N.E., les chercheurs du Centre Géoscientifique de l'Atlantique emploient régulièrement plusieurs méthodes pour désagreger les échantillons de sédiments utilisés pour l'analyse des microfossiles coquillés tels que les foraminifères et les ostracodes. Cette publication décrit et illustre ces méthodes, dans le but de les enseigner aux étudiants sans expérience. Y sont joints également une brève liste de matériaux et une bibliographie sélectionnée des publications traitant de ces techniques ainsi que d'autres.

AUTHORS' NOTE

What motivates the compilation of another processing techniques paper? It is the authors' belief that there is a need for a small, portable compendium of these methods.

Textbooks presently used in university micropaleontology courses often contain an overall survey of these techniques, but do not offer the student the clear step-by-step directions often needed in laboratory work. This handbook has been written with a view towards guiding even the most inexperienced student safely through the steps involved with a minimum of problems.

Common sense, of course, is an important ingredient in any laboratory work, and the users of this manual will, with practice, soon find the most efficient way to do a given procedure, saving time and effort along the way.

The authors wish to encourage readers, to experiment on their own with processing techniques and would like to hear about any improvements or variations of these methods or any others which prove successful, so that they may be included in a later edition of this work.

The thought that provoked the authors' participation in this project is that nothing is absolute and it all needs revisions time and time again. We only hope to promote a more conscious attitude in your approach to 'day to day' routine.

Finally, the authors wish to express appreciation to F.M. Gradstein, A.G.C., for many useful suggestions during the compiling of this manuscript, and to F.E. Cole and M.A. Williamson, both of A.G.C., for critically reviewing the final text. G. Cook, A.G.C., vastly improved and drafted the authors' original diagrams.

TABLE OF CONTENTS

1.0 INTRODUCTION

This handbook was compiled to provide the student of micropaleontology with a general guide to some of the more common methods of preparing rock or mud samples for analysis of calcareous microfossils, particularly foraminifera and ostracodes.

It is limited to those methods actually used by the authors, but includes all the procedures used by researchers at the Atlantic Geoscience Centre and COGLA (Canadian Oil and Gas Lands Adminstration), both located at the Bedford Institute of Oceanography.

All the methods described herein are basically simple, and require only the equipment found in most laboratories, such as ovens, hot plates, and other common items.

Schematic diagrams (Figs. 1-6) are included with the description of each method, and provide the reader with a simplified visual guide to the steps in the procedure.

Some of the procedures require the use of potentially dangerous chemicals such a solvents and acids, and readers inexperienced in the use of these substances are urged to seek supervision or advice before attempting these methods.

As a general rule, the processing method used for each sample or suite of samples is dictated by the nature of the sediment itself; for example, a method good for limestones may be inappropriate for use with soft muds. Table I provides the reader with a quick guide to choosing the disaggregation procedure best suited for the type of sample to be processed. In some cases, of course, one process may not work satisfactorily on a given sample, in which case it may be necessary to try a different method, or repeat the first one until enough material has been broken down

TABLE 1

Processing Method

	Acid Treatment	Calgonite Method	Freeze Drying	Industrial Soap	Oxidation Method	Solvent Method
Hard limestones	Yes		May work	May work		May work
Softer limestones	Yes		Yes	Yes		Yes
Hard shales, siltstones, sandstones			Yes	Yes		May work
Softer shales, siltstones, sandstones			Yes	Yes		
Black, carbonaceous shales or siltstones		May work	Yes	Yes	Yes	
Recent or unconsolidated muds or silts		Yes		Yes	May work	

Table 1. Indicates which method(s) may be most suitable for various sample types.

to allow analysis. Normally, the residues from these methods are washed using a 63 micron (#230) sieve.

Near the end of this handbook, the "Selected References" chapter provides the user with a brief list of some other material dealing with methods of preparation of samples. This is not a complete listing, but is designed to furnish additional information on techniques not detailed in this handbook. Finally, a brief list of suppliers of chemicals and equipment needed for these procedures is provided.

1.1 Complications

There are several small problems which may arise when processing samples for micropaleontological analysis by any method. These complications are best discussed before any procedures are begun.

The first difficulty that one may encounter is in heating the samples. Ovens should be set for no more than 40°C (104°F) in order to avoid baking the clay portion of samples, particularly in Recent, unconsolidated, material. If this occurs, the sample becomes quite hard, and is correspondingly more difficult to disaggregate.

An ultrasonic bath for further disaggregation of sediment should preferably be used in those cases where tests of organisms have become silicified or otherwise hardened, as in many Mesozoic and Tertiary sediments. The unaltered, relatively delicate tests of ostracodes and foraminifera in unconsolidated material may be damaged or destroyed by exposure to prolonged ultrasonic vibration, but short periods of treatment are an excellent means of "shaking off" cohesive deep sea clays from the coarser microfossil tests.

When using the "Calgonite" or "Industrial Soap" methods, the pH of

the soaking samples should be checked periodically, and sodium carbonate added where necessary to keep the pH above 7 and thereby prevent acid etching and damage to tests.

2.0 ACID TREATMENT

2.1 Introduction

This is a highly unusual method of disintegrating hard limestone samples for micropaleontological study, involving the use of concentrated acetic acid. The method first came to the attention of the authors in an unreferenced, typed manuscript by Tilo Notzold (1965).

In this procedure, the sample is immersed in concentrated (glacial) acetic acid, along with a small amount of dehydrated copper sulphate. The presence of copper sulphate causes the acid to weakly dissociate, which will result in the acid attacking the limestone more quickly than it affects calcareous fossils such as foraminifera and ostracods. This dissociation is caused by the hygroscopic sulphate absorbing moisture from the atmosphere.

It is most important, however, that this dissociative process remains weak; if too much humidity is available, the process increases in strength and fossils are attacked as quickly as the surrounding rock.

There are several major disadvantages to using this method. The first is that it is a very slow process, taking weeks or even months to complete. Secondly, it can be difficult to judge just how much acid to use, and fossils can be lost or damaged. Furthermore, the sample in the acid bath should be kept in a fume hood for the entire duration of the experiment. Lastly, compounds such as concentrated acetic acid and ammonia are potentially dangerous and always have to be handled with great care.

On the positive side, the method does break up large chunks of hard limestone into smaller bits quickly, and requires little equipment.

2.2 Materials

Concentrated Acetic Acid (Glacial)

Anhydrous copper sulphate (if only hydrous copper sulphate is available, water of hydration can be driven off by heating in an oven for 2-3 hours)

Glass Beaker

Fume Hood

(NH_4OH)

Ammonium hydroxide

2.3 Procedure

1. Place sample (ideally a piece of limestone 3-5 cm in diameter) in glass beaker, (Fig. 1).
2. Add 200 - 250 ml concentrated acetic acid.
3. Add 15 - 20 g anhydrous copper sulphate.
4. Cover loosely (to allow for some air exchange) and place in fume hood.
5. Length of time it takes for complete breakdown is dependent on the hardness and porosity of the sample. The authors have experimented with this method and have found that after twenty-one days a comparatively soft limestone has disintegrated rather well. Notzold (1965) reports a period of 30 - 40 days as ideal for obtaining foraminifera and ostracods.
6. The samples should be checked visually every other day or so, and certain signs should be watched for.

   Formation of small gas bubbles on the sample indicates that the dissociation process is too strong. There may also be an excess of calcium acetate crystals formed during the reaction. This can be

remedied, according to Notzold, by the addition of more acetic acid. Another problem which may arise is the entire sample congealing because of the very high freezing point of concentrated acetic acid (15°C). If this occurs, the sample can be thawed by restoring the temperature to more normal room values.

7. The entire acid disaggregation method can be stopped at the discretion of the individual, and judging when to do this is a somewhat subjective decision. In general, do not leave it until all the limestone is dissolved, or destruction of at least some of the fossil material is highly likely.

   To stop the process, pour off the excess acid, and slowly add a small quantity of 25% ammonia (NH_4OH) immediately (in the fume hood). A fairly strong reaction will take place - (try to avoid splashes on your skin). After 2-3 hours, the sample can be washed to dissolve excess salts and remove finer material.

8. After drying, any larger bits of undissolved limestone may be reprocessed the same way, if desired. It has been our experience, however, that even the larger pieces will have been sufficiently softened and weakened to be broken down in one or another of the quicker methods described elsewhere in this handbook.

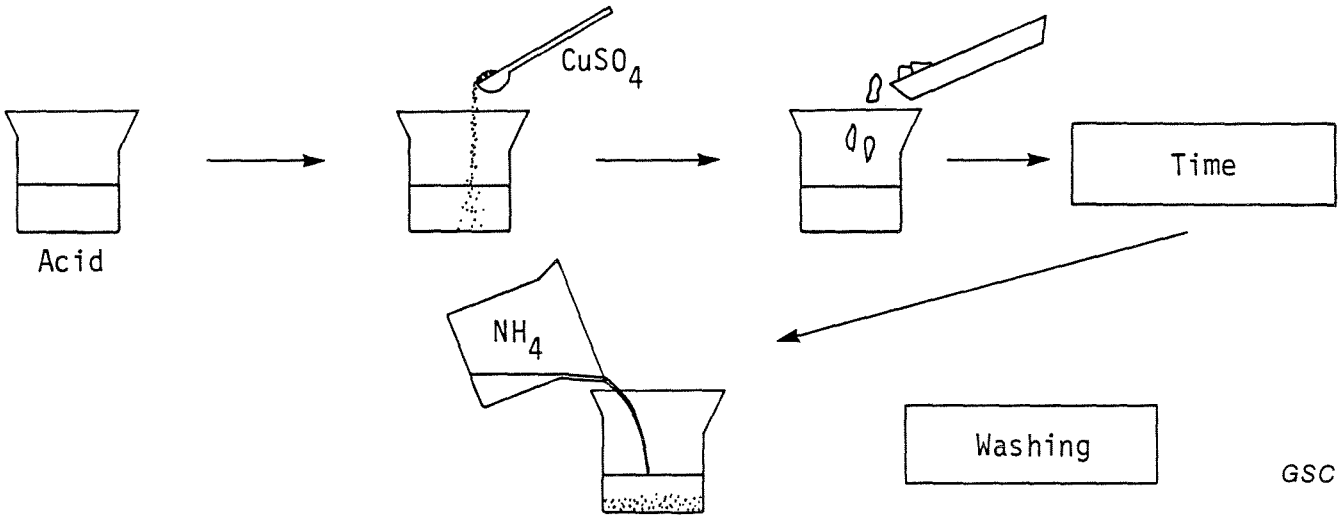

Fig. 1 Schematic drawing of steps involved in Acid Treatment.

3.0 CALGONITE METHOD

3.1 Introduction

"Calgonite" is a readily available commercial household product commonly used to soften water for baths and laundry. Its value in micro-paleontological studies lies in its ability to soften and disaggregate weakly indurated shales or softer, Recent sediments which have been allowed to dry out.

This method reuires little equipment and is relatively fast, but does not work well with limestone or harder shales.

If Calgonite is unavailable, a small quantity of some other product such as Fisher Scientific's "Versaclean" is a suitable substitute.

3.2 Materials

40-dram plastic snap-cap vials

Quantity of Calgonite, Versaclean, etc.

Distilled or otherwise purified water

Sodium Carbonate

Kettle

3.3 Procedure

1. Samples should be placed in 40-dram vials (Fig. 2). (If sample is Recent mud which has dried out, or soft shale, it should be heated. If it is wet mud, use as is).
2. Small quantity (2-3 g) of Calgonite added. (If using Versaclean or other liquid agent, make up a 30:1 solution and use 5-10 ml).
3. Add boiling water.
4. Test pH, sodium carbonate may be added if necessary.

5. Samples should be shaken briskly by hand ocasionally during the next 24 hours (keep a thumb or two fingers on the cap; sometimes it comes off during the shaking, resulting in lost sample and messy clothes).
6. After 24-48 hours, the samples should be ready for sieving.

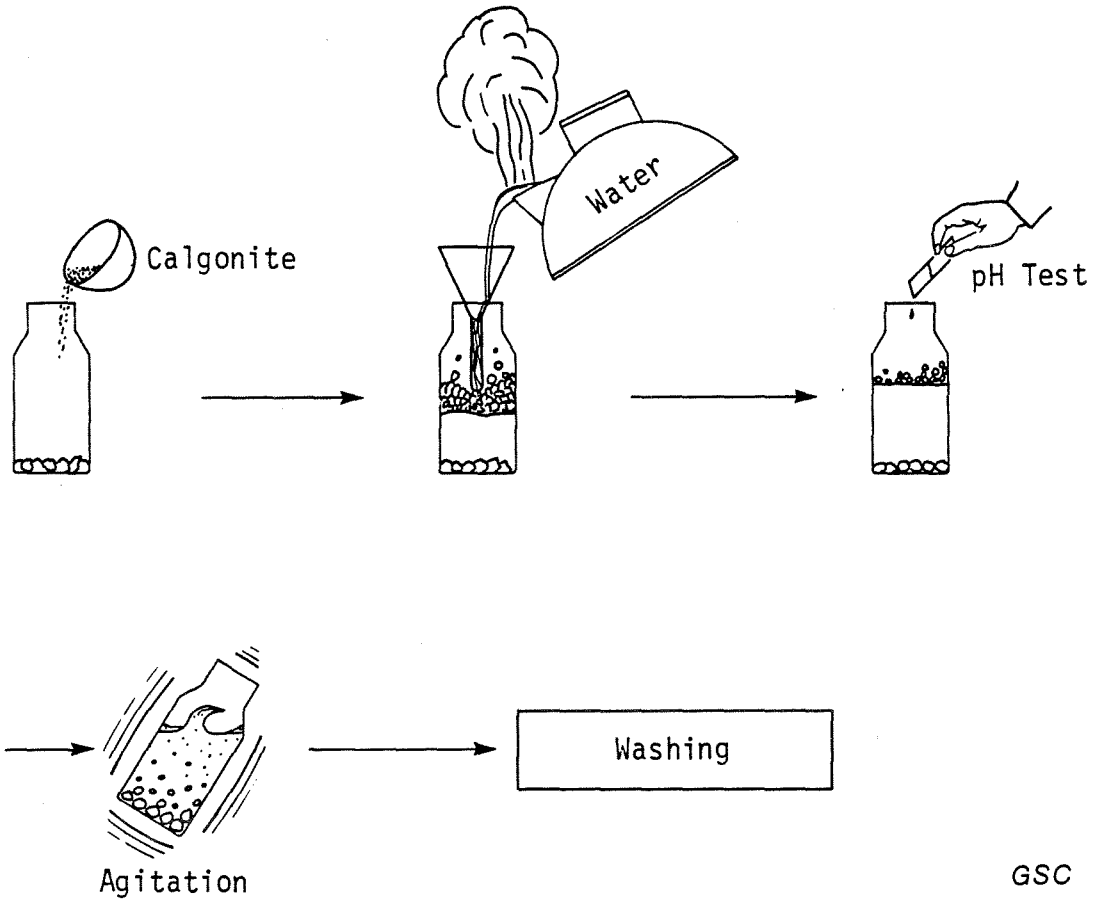

Fig. 2. Schematic drawing of steps involved in Calgonite Method.

4.0 FREEZE-DRY METHOD

4.1 Introduction

This method is very suitable for hard, indurated samples, where shelly microfossils such as foraminifers are not particularly fragile.

It is reasonably fast and requires only some distilled water, a freezer and a vacuum bell jar.

It is also sometimes used to dissaggregate organic-rich muds preparatory to "ashing" them for sedimentological studies.

4.2 Materials

Distilled water

Freezer (minimum temperature -20°C)

Vacuum Bell Jar

4.3 Procedure

1. If sample is dry to start, soak for a few hours in distilled water to thoroughly saturate it (Fig. 3).
2. Excess water can be decanted off, and sample placed in freezer.
3. After four or so hours, the sample may be removed from the freezer and placed directly in a bell jar, which is then evacuated.
4. As the water in the sample sublimates, pressures are built up and some crumbling should occur.
5. The whole process may be repeated as many times as necessary.

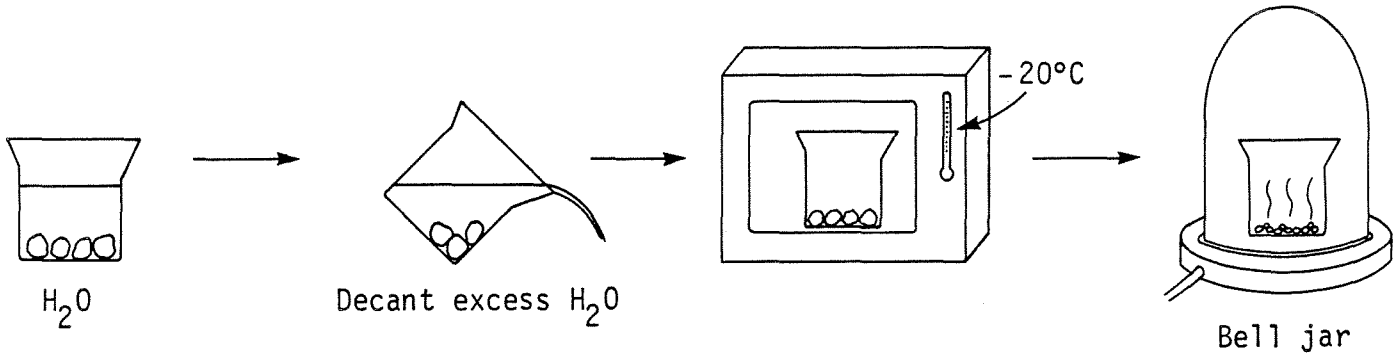

Fig. 3 Schematic drawing of steps involved in Freeze-Drying Method.

5.0 INDUSTRIAL SOAP METHOD

5.1 Introduction

The single most commonly used method for disaggregating indurated drilling samples (e.g. shales, siltstones, mudstones) at COGLA involves the use of a heavy industrial soap or wetting agent such as Ciba - Geigy's "Quaternary O" or Domtar's "Miramine OC-ES". Peterson et al. (1983) provide a list of possible alternatives to these substances, along with comparisons of their effectiveness in experiments.

In this process the sample is soaked for a period of one to four or more days, depending on its initial hardness, in a solution of soap and water. At some point during the soaking period the mixture may be subjected to simultaneous heating and stirring, (as on an oscillating hot plate) and normally is treated with 2-5 minutes of ultrasonic vibration.

This method works reasonably well with most clastic sediments providing that they are not very hard, but is somewhat less successful with limestones, particularly hard ones. In any case, for best results the sample should first be reduced to fragments no larger than 1 or 2 cm diameter before processing. This can be done with a hammer, mortar and pestle, or motorized crusher. Some fossils may be damaged or detroyed, but most will survive.

The one real disadvantage of this method is that it requires some equipment such as oscillating hot plates and materials like Quaternary O which may not be available in some laboratories.

5.2 Materials

Miramine OC-ES or Quaternary O

Distilled or otherwise purified water

Stainless steel 600 ml beakers

Glass stirring rods

Sodium Carbonate (powdered)

pH test paper

Drying oven

Oscillating hot plate

5.3 Procedure

1. Sample should be no more than 200 gm dry weight, and no fragments should be larger than 1 or 2 centimeters in diameter. If necessary, crush using a mortar and pestle, or hammer.
2. Sample should be thoroughly dried and heated in an oven (Fig. 4).
3. Prepare a soap solution by diluting 1:3 with distilled water.
4. Boil more distilled water.
5. To the stainless steel beaker, add 80-100 ml of soap solution, approximately 200 ml boiling water, and promptly drop preheated sample in.
6. Stir with glass rod.
7. Test pH; if at all acidic, add a small amount of sodium carbonate (0.5 g is usually sufficient).
8. At some point during the next 1-4 days, the beaker should be placed on an oscillating hot plate and lightly boiled. (Oscillation is most important, both for its mechanical effect in abrading small bits of sample, and in that it helps to prevent the mixture from burning on to the bottom of the beaker). If no oscillating hot plate is available, the sample should be stirred frequently during heating. One and one-half to two hours on the hot plate should be sufficient. Care should be taken to add more water to the samples to prevent drying out should

it become necessary.

9. Beakers should be placed in an ultrasonic bath for 2-5 minutes for additional disaggregation, after the heating treatment.

10. It is a good idea to retest the pH after the heating treatment, because sometimes quantities of new material are liberated by the agitation, and may lower the pH. If this occurs, addition of a little more sodium carbonate should remedy the situation.

11. Two to four days after beginning this treatment, the sample should be ready for washing. In many cases, much of the sample will still consist of relativley large fragments, and where this occurs a repetition of the entire process is suggested.

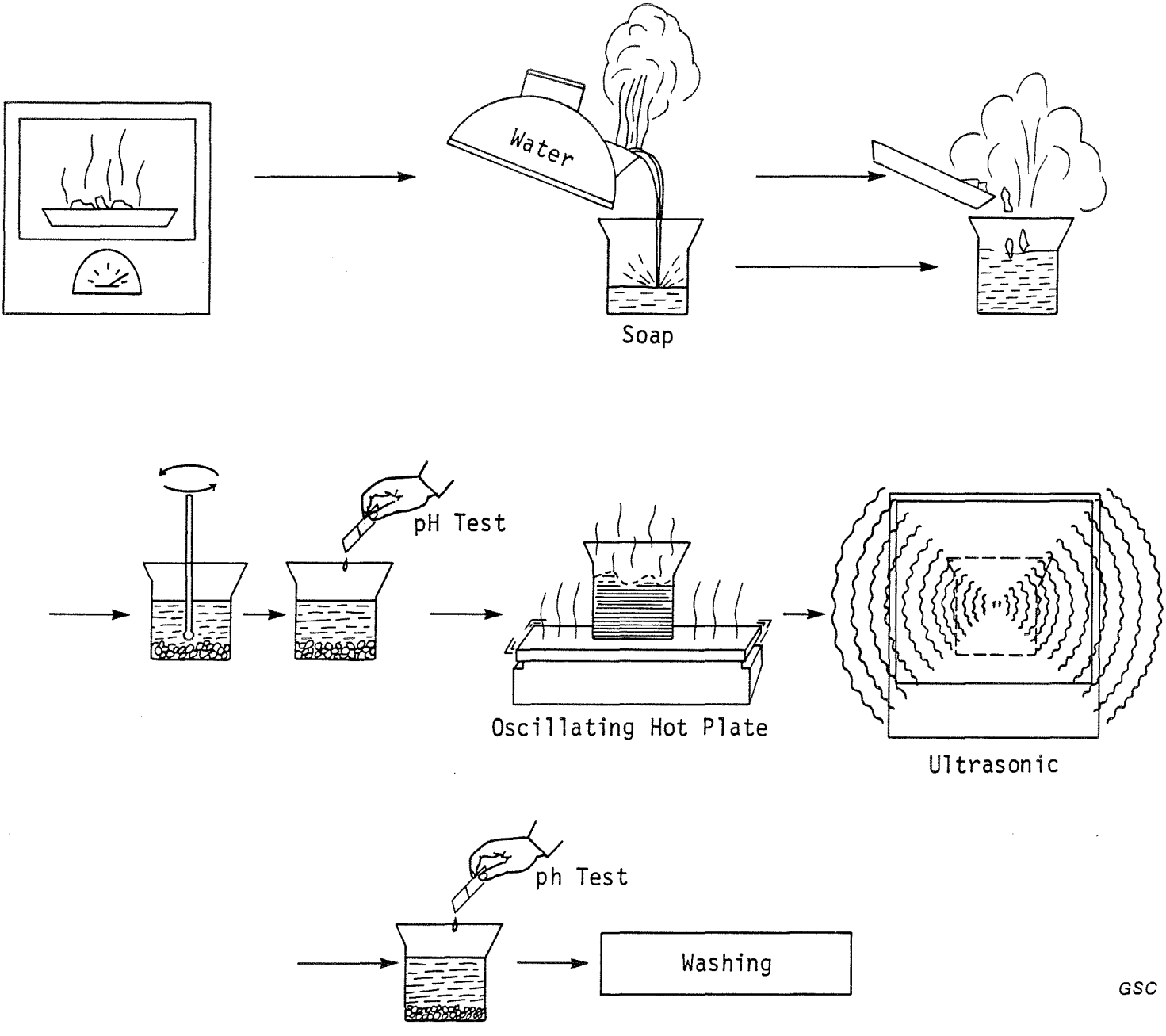

Fig. 4. Schematic drawing of steps involved in Industrial Soap Method.

6.0 OXIDATION METHOD

6.1 Introduction

In this procedure, the strongly oxidizing powers of hydrogen peroxide are utilized by the micropaleontologist.

Black shales or mudstones rich in organic material can often be induced to disintegrate by adding hydrogen peroxide and subjecting to heat.

Little equipment is necessary for this procedure, and it can be done quite quickly.

NOTE: CONCENTRATED HYDROGEN PEROXIDE CAN BLEACH SKIN OR ANYTHING ELSE IT CONTACTS. USE CAUTION AT ALL TIMES WHEN HANDLING IT.

6.2 Material

Hydrogen peroxide 30%

Beakers (Steel or pyrex)

Distilled water

Hot plate (preferably oscillating)

Fume Hood

6.3 Procedure

1. Combine sample and some distilled water in a beaker (Fig. 5). (Three parts water to 1 part sample).
2. Add a small amount of concentrated hydrogen peroxide (10 ml to a 400 ml sample/water mixture should be enough).
3. Place on hot plate and bring to a low boil. If you do not have an oscillating hot plate, stir frequently.
4. Stand by; when the hydrogen peroxide begins to work, the whole sample

may begin to boil quite violently. If this happens, remove from heat immediately, since excessive boiling may damage microfossil tests.

5. When the samples begin to boil, lower the heat and leave them for an hour or so.

6. After this the samples may be ready for washing; or, if not, the process may be repeated.

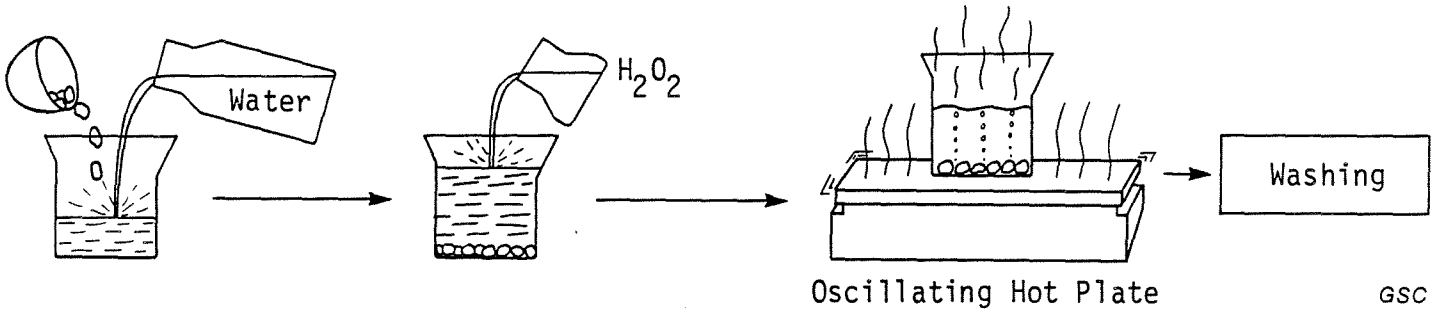

Fig. 5. Schematic drawing of steps involved in Oxidation Method.

7.0 SOLVENT METHOD

7.1 Introduction

This is a simple method of disaggregating very hard sediments for micropaleontological study, and requires relatively little time or equipment.

This treatment is especially useful as a method of reducing large chunks of sample to more manageable fragments if one does not wish to resort to a mortar and pestle, which could damage larger foraminifera and other fossils. Various solvents can be used in this procedure; in general one should look for a material that is:

1. Not too inflammable.
2. Not too toxic.
3. Readily available.

Some suggested materials are:

1. Methanol
2. Methyl hydrate (paint thinner)
3. Turpentine
4. Kerosene

NOTE: ALL SOLVENTS SHOULD BE TREATED WITH CARE. HANDLE ONLY IN FUME HOODS, AND KEEP AWAY FROM OPEN FLAME OR SPARKS. OBVIOUSLY, SMOKING IS ESPECIALLY UNWISE WHILE HANDLING SOLVENTS.

ETHERS, ESPECIALLY PETROLEUM ETHER, ARE NOT RECOMMENDED. THEIR LOW FLASH POINTS MAKE THEM ESPECIALLY HAZARDOUS TO WORK WITH.

7.2 Materials

Solvent

Distilled water

Kettle

Beaker (Pyrex or steel)

Fume Hood

7.3 Procedure

1. Sample should be placed in oven and thoroughly dried and heated (Fig. 6).
2. Put sample in beaker. Under the fume hood, pour in solvent to cover.
3. Loosely cover beaker and place in fume hood.
4. After 30 minutes - 8 hours, pour off solvent (this may be filtered and re-used).
5. Immediately pour boiling distilled water onto sample to cover.
6. The interaction of the hot water and solvent-soaked sample should produce a "fizzing" or even a "popping" effect, as the sample partly disintegrates. After half an hour or so, the liquid can be decanted off, and the sample may be washed if sufficient disintegration has taken place. If not, this method may be repeated as many times as necessary.

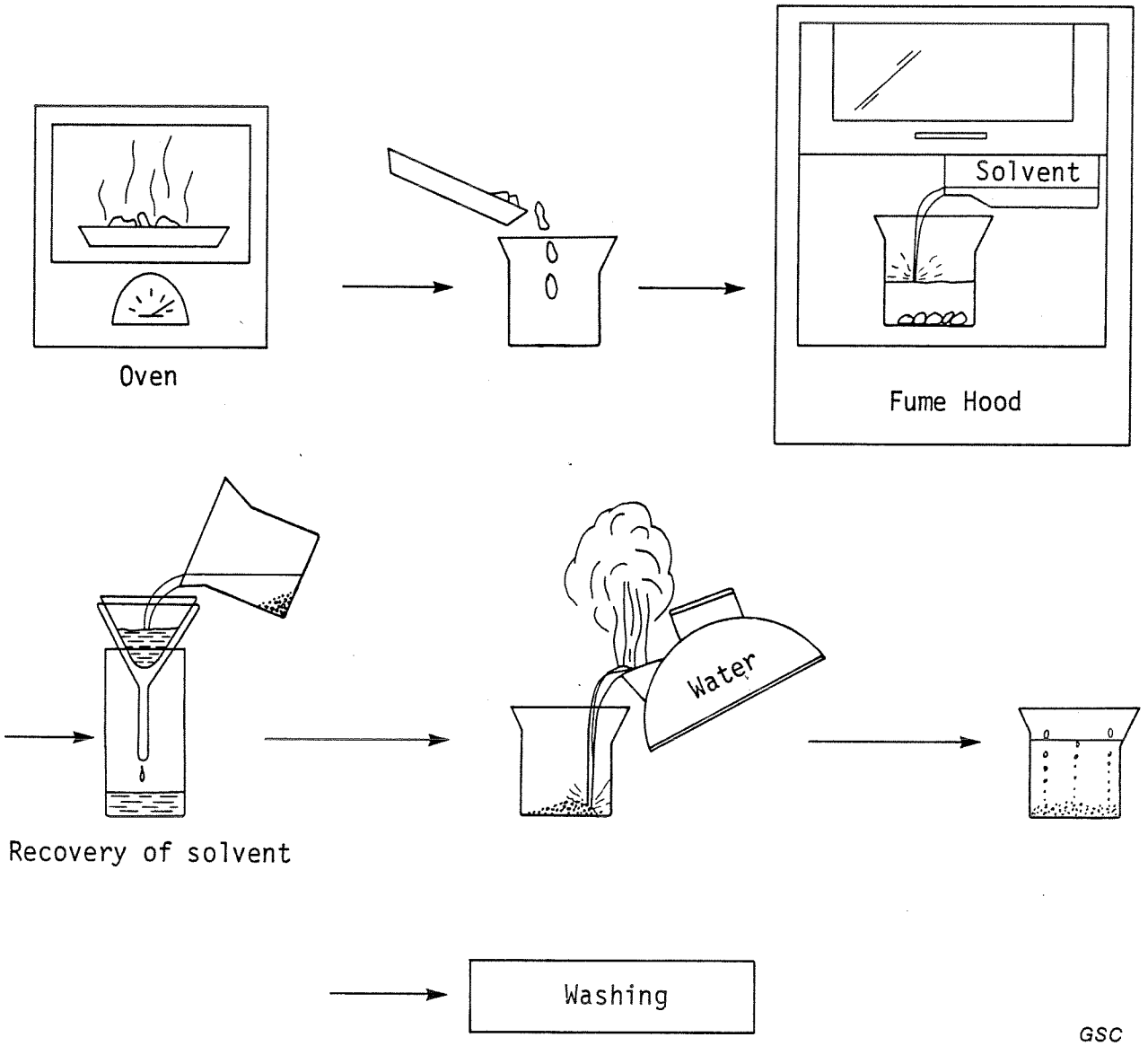

Fig. 6. Schematic drawing of steps involved in Solvent Method.

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9.0 MATERIAL SOURCES

Canlab
10 Morris Drive
Suite 12, Building A
Dartmouth, Nova Scotia
B3B 1K8
(902) 463-8270

- beakers, chemicals, hot plates, ovens, miscellaneous items

Fisher Scientific
18 Morris Drive
Dartmouth, Nova Scotia
B3B 1K8
(902) 469-9891

- beakers, chemicals, hot plates, ovens, miscellaneous items

Domtar Chemicals Group
Organic Chemical Division
104 Doyon Ave.
Pointe Clair, Quebec
H9R 3T5
(514) 697-4240

- Miramine OC-ES