ABSTRACT

Two methods of chemical concentration of diatoms occurring in fresh-water sediments, and one chemico-mechanical method of concentration for diatoms occurring in marine sediments are outlined, with emphasis on the extreme care that needs to be exercised during the preparation of samples. A method for the preparation of strewn slides is then explained. A detailed procedure for the study of diatom-strewn slides under the microscope, using the "England finder", is described, and a note on diatom analysis is added.

Preparation and method of study of fossil diatoms

M. G. ANANTHA PADMANABHA SETTY
Department of Geology
Bangalore University
Bangalore, India

INTRODUCTION

A long and time-consuming process is required to prepare a slide of diatoms from a field sample. The preparation techniques of fossil diatom slides vary with the kind of material. If the material is consolidated or loosely consolidated, and consists of late Tertiary or Quaternary lacustrine, estuarine or lagoonal fresh-water sediment which is rich in organic matter, chemical concentration methods are the most suitable. However, if the material is chiefly marine, and the amount of organic matter is negligible, but lime and colloidal clay interfere with the concentration of diatoms, chemico-mechanical methods are to be adopted. Several methods have been proposed by different authors (Munthe, 1894; Olsson, 1929; Halden, 1929; Hustedt, 1958), but the following method, adopted from Kanaya (personal communication), is used by the author, who found it suitable and extremely efficient in his study of the fossil diatoms of Lake Bonneville, Utah. In addition to this, two other methods are also described.

PREPARATION OF SAMPLES

Method no. 1

1) First describe the lithology of the sample. In addition to field notes, describe the lithology fully and completely (including its color, cohesiveness, porosity and mineralogy – both megascopic and microscopic study of minerals, their identification, grain size, shape, association and other characteristics, if any; and the kind and nature of the matrix, the associated fauna, its mode of preservation, etc.). It is of prime importance to study each sample separately so as to avoid contamination in the laboratory as well as in the field.

2) Dry the sample overnight at 125°C.

3) Cool the sample to room temperature, and then take 200 mg. of the sample in a 250 cc. hard glass pyrex beaker, treat it with c.a. 15% concentration of hydrogen peroxide, and boil for 20 minutes on a hot water bath. Hydrogen peroxide breaks up the solids and disperses them into particles, while the organic matter is oxidized and destroyed. A larger sample of about 500 mg. is recommended if the material is poor in diatoms.

4) After boiling, fill the beaker with distilled water (distilled water alone to be used throughout) and allow it to stand for 24 hours.

5) Decant the supernatant liquid very carefully.

6) Add c.a. 25% concentration of hydrochloric acid to the residure and boil again for 15 minutes, at the end of which any calcium salts will be dissociated.

7) Cool, and, after cooling, fill the beaker with distilled water and allow it to stand for 24 hours.

8) Decant the suspensions very carefully, fill the beaker with distilled water again, and allow it to stand for 24 hours.

9) Again decant the suspensions very carefully and add c.a. 25% concentration of nitric acid to the residue, whereby the last traces of organic matter are destroyed, and other inorganic salts are also removed. Then, fill the beaker with distilled water and allow it to stand for 24 hours.

10) Decant, carefully transfer the residual sample into a larger beaker of 1.5 L. capacity, and fill the beaker with distilled water so as to make the water column 10 cm. in height. (A particle having a hydraulic diameter corresponding to a quartz sphere of 1 mm. in diameter sinks approx. 8.5 cm. in 24 hours in water at room temperature.)

11) Decant carefully, fill the beaker with distilled water as in Step 10, and allow it to stand for 24 hours.

12) Repeat Step 11 four more times.

The residue is now free from all organic matter and soluble minerals. It consists of diatoms (together with pollen and spores, and any other acid-resistant fossils) with the finer clastic sediments in suspension, and represents only a fraction of the original material. Now the residue is ready for making slides.

If need be, the diatoms and the associated microfossils can be separated from the clastic sediments with the aid of a centrifuge. "Calgon" (sodium hexametaphosphate), soluble in water and having a pH of 9 to 9.5, is used as a dispersing medium.

Method no. 2

Another chemical concentration method is as follows:

1) After Steps 1 to 5 of Method no. 1, treat the residue with concentrated hydrochloric acid, warm it for 15 minutes, and allow it to stand for 3 days.

2) Then decant, add distilled water, and allow it to stand for 24 hours.

3) Repeat Step 2 above 8 to 10 times.

4) Decant, add chromic acid, and allow it to stand for 3 days.

5) Then decant and repeat Step 4 five times.

6) Decant, add distilled water, and allow it to stand for 24 hours.

7) Repeat Step 6 above 6 to 8 times.

8) Decant and store the residue in 70% butyl alcohol.

Now the residue is ready for making slides.

Method no. 3

In the case of marine clays in which there is a small quantity of organic matter, besides lime and other salts with fine-grained clastics in addition to colloidal clay, chemico-mechanical methods are used. One of the procedures is as follows:

1) Follow Method no. 1 from Step 1 to Step 9.

2) To eliminate the colloidal clay, fill the beaker with dilute ammonia solution and allow it to stand for 6 hours.

3) Decant the suspensions and repeat Step 2 above 15 to 20 times.

4) Add distilled water and allow it to stand for 24 hours.

5) Decant and repeat Step 4 four times.

Now the residue is ready for making slides.

PREPARATION OF SLIDES

Preparation of slides for microscopic examination of diatoms is of two kinds: 1) preparation of oriented slides of single species, and 2) preparation of strewn slides.

Although oriented slides of single species are of great value for a critical taxonomic study, in view of the extreme smallness of the diatoms, special techniques are required in their preparation in addition to extreme patience and time. Hanna uses a mechanical finger mounted on the stage of the microscope for preparing the oriented slides (personal communication). Olsson (1929) and Hustedt (1958) have described in detail the techniques of preparation of diatom slides. The writer, however, chose to confine his studies to strewn slides, and the technique of their preparation is detailed below.

1) The residue obtained after Step 12 (Method no. 1) and Step 8 (Method no. 2) in the case of fresh-water diatom samples and after Step 5 (Method no. 3) in the case of marine clays or sediments is diluted with distilled water to make 40 cc. and stirred well so that the diatoms and clastic particles are brought into suspension.

2) Then, using a pipette, take 0.5 cc. of the suspension, consisting of diatoms, other microfossils, clastic sediments and water, and deposit it evenly on a cover glass. This amount represents 2.5 mg. of the original rock, provided that the original rock is completely disintegrated, and is considered as a 1-unit thickness. Now allow it to dry. This can be hastened by warming the cover glass on a hot plate. If the material is highly diatomaceous, even $\frac{1}{4}$ unit is sufficiently thick. On the other hand, if the material is poorly diatomaceous, it can be increased to a 2-unit thickness.

3) Now, put a drop of a mounting medium, such as Hyrax, Canada balsam, Caedax – recommended by Hustedt in 1958 (Miller, 1964) – or Clarite, on this dried sample, let it warm up to 250° C, and cover it very cautiously with a standard glass slide. Any air bubbles that may form are eliminated by applying a little pressure on the slide.

4) Now, let the slide cool and wash it with xylol to remove the excess mounting medium. Then wash the slide with soap, rinse in distilled water and dry.

5) Finally, the slide is labelled with the required data, preferably to the left of the cover glass.

Now the slide is ready for examination under the microscope. A minimum of 4 and a maximum of 10 strewn slides are prepared for each sample. The unused remainder of the residue is placed in a small vial, immersed in butyl alcohol, and stored with proper labeling for any future use.

STAINING

Fossil diatoms, by virtue of the chemical composition of their frustules ($SiO_2.nH_2O$) do not usually pick up any stain. The writer, however, tried stains like methyl blue, fuchsin (basic dye), safranin – Y and safranin – O; methyl violet and thymol blue. A very small quantity of the stain was added to the residue after Step 9 (Method no. 1). Nondiatomaceous microfossils which

readily picked up the stain showed a high resolution and greater contrast when viewed under the microscope. The slides which were treated with methyl blue gave a high degree of contrast and hence were very useful in photomicrography.

METHOD OF STUDY

A detailed method of study of diatoms in a strewn slide does not seem to have been recorded by any previous worker. However, it is known that Van Huerck, in 1893, used a Maltwood finder (Kanaya, 1959). As this finder is not available in the market, Kanaya described the method of its preparation. However accurately one may prepare a Maltwood finder of one's own, these home-made finders will differ from one another to some slight degree and thus lead to difficulties. This problem was overcome by the introduction of the England finder (manufactured by Graticules Ltd., London). This England finder, which has the dimensions of a standard glass slide, consists of a grid of 60 squares in each horizontal row, each square being divided into four numbered quadrants, and 26 rows arranged in alphabetical order, one below the other. The Maltwood finder is very similar to this except for its quadrants.

With the help of this England finder it is quite easy to record the position of any diatom in a strewn slide very accurately. This information becomes very useful in any future photomicrographic work and also as a reference to the desired specimen. The position of the desired diatom in any strewn slide is recorded as: Name of the diatom species, Slide number (X), M26, Q 3 (with reference to the letter, the number and the four quadrants of the square in the grid). See text-figure 1.

The following is the procedure in this respect:

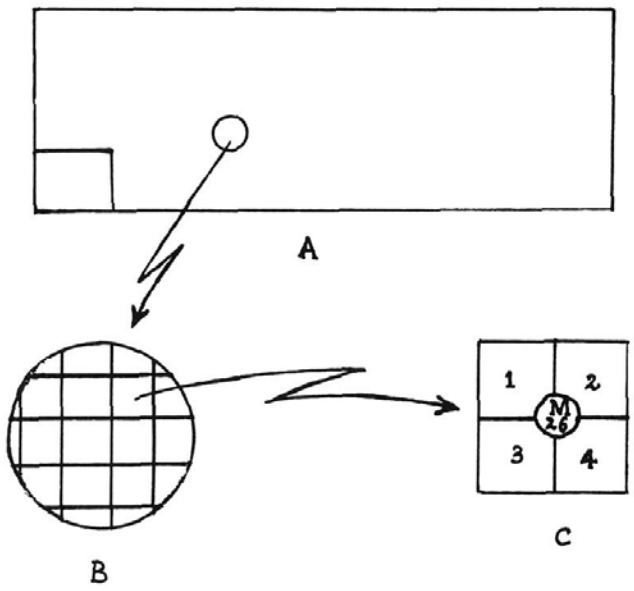
1) First, mount the England finder on the mechanical stage of the microscope. (Under proper focus and at low magnification the grid in the England finder shows several rows of squares, each square with its letter, number and quadrants.)

2) Then place the strewn slide under study on top of the England finder. By raising the stem of the microscope, the diatoms present in the strewn slide can be brought into the field of vision.

Thus, by very careful manipulation of the stem of the microscope either the England finder or the strewn slide can be brought into view, while both are in position on the stage.

3) Now, in order to make an accurate record of the position of the desired specimen in the strewn slide, move the desired specimen (after its identification, if necessary) to the center of the field of vision or to the cross hairs (as the case may be) under proper focus.

4) Then bring the England finder into focus by lowering the stem of the microscope carefully, and locate the



TEXT-FIGURE 1

A, England finder (actual size); B, the grid under low magnification; C, one of the squares in the grid showing the letter, the number and the quadrants under high magnification.

letter, the number and the quadrant of the square on the grid, situated exactly beneath the center of the field of vision or the cross hairs. Now the desired specimen on the strewn slide corresponds exactly to a particular square – the letter, the number, and one of the quadrants in the grid which is located immediately below it.

5) Write down the position established as a record for future guidance. The previously cited example would appear as: Name of the specimen, Slide number (X), M26, Q 3.

By a number of horizontal or vertical traverses the entire slide is scanned, all the different species of diatoms, pollen, spores, spicules, desmids, mineral grains, *etc.* are recognised, and the desired specimens are recorded as to their location with the help of the England finder.

DIATOM ANALYSIS

Both quantitative and qualitative analyses of diatoms in sediments are made for purposes of stratigraphic and paleoecological studies. A quantitative analysis involves physical counting of at least 200 individual diatom frustules per slide or at least 2,000 specimens per sample, by species. Sometimes, as a result of chemical and chemico-mechanical processes, the frustules break apart into valves (hypovalves and epivalves). Under such circumstances, each valve should be counted as one unit, and a complete frustule as 2 units. The counting and identifying of diatoms is done as they appear

along the lines of traverse for purposes of study of frequency, relative abundance of the species, their distribution throughout the sequence, and their density through the different samples. The relative abundance and the frequency of diatoms is then estimated and the statistics recorded in the distribution chart, histograms, and frequency and cumulative curves. A qualitative analysis presents a percentage distribution of diatoms, which in turn gives a ratio between the planktonic and the benthonic species, and a percentage distribution between diatoms and other fossil assemblages.

A variation in the kind and number of diatoms in the stratigraphic scale indicates the fluctuation in the number of those diatoms. A word of caution that is to be taken into cognisance here is that the concentration of a particular diatom may vary very much between two different samples from the same level. These lateral changes in frequency may chiefly depend upon the local conditions of sedimentation.

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