THE CHEMICAL COMPOSITION OF THE DIATOM FRUSTULE

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Introduction

It is well known that the frustule or the wall of diatoms is siliceous. But the exact chemical composition and the physical condition of the silicate present is not quite clearly known at the present time.

Kützing (1844), Ehrenberg (1834), Bailey (1856) and Weiss (1871) found that SiO_2 was present in the frustule, and for a long time it was thought that SiO_2 was the only constituent. Subsequently definite evidence has been obtained that SiO_2 constituted up to as much as 87-99% of the ash, and that a smaller portion (up to 2%) was made up of ferrous and aluminium oxides. Some other elements are also found in traces [*see* for instance Kützing (1844), Samoilov and Roskhova (1925), Rogall (1939), Vinogradov (1953), Desikachary (1956), also Murray and Penard (1891), Kützing (*ex* Ehrenberg, 1834), Naumann (1930), Richter (1911), Sjostedt (1921) and Tschirwinsky in Novotscherkesk (1925)]. Estimations have shown that the SiO_2 content varies with the individual species and with the environmental conditions in which they grow [Pia (1926), Rogall (1939), Einsele and Grim (1938)]. The centric diatoms as a rule are found to have a lesser quantity of silica than the pennate forms.

The silica in the diatom frustule is considered, in recent times, as amorphous [Kahane (1935), Rogall (1939), Vinogradov (1939)]. According to Liebisch (1929-30) the silicate in the frustule is in the form of hydrated silica $SiO_2.xH_2O$ very similar to opal; while Cooper (1952) is of the opinion that the chemical constitution of the diatom skeletons is similar to that of hydrated silica dispersed in water. Rogall (1939) concluded from his studies that the frustules were made of pure silicic acid in a sub-colloidal state. The

difficulty arises in correlating the above observations on the present-day diatoms with that of the crystalline condition of the silica seen in the fossil diatoms [Rogall (1939) and Geissler (1958)]. There are two ways of explaining this. It is possible that in both the recent and the fossil diatoms silica is present in a crystalline condition, but that the crystalline pattern is obscured by the diffuse diffraction pattern due to other material such as protein or pectins in the present-day diatoms. The other explanation is that the deposition is in an amorphous condition and that only during fossilization there is a rearrangement of the molecules leading to a progressive crystallisation of the silica. In other words, the latter assumes that the silica is transformed during fossilization of the diatoms from an amorphous to a crystalline condition.

The fact that the silica in the present-day diatoms is in an amorphous condition was deduced by the earlier workers from their isotropic behaviour since they are optically non-birefringent and also because there is a negative membrane potential. The absence of any distinguishable crystalline pattern in diffraction studies also confirmed this idea. The fossil diatoms on the other hand yield a diffraction pattern characteristic of a crystalline condition, and have a much lower membrane potential than the present day diatoms (Geissler, 1958).

These X-ray diffraction studies on the diatom frustules were carried out by Rogall (1939) who seems to be the only one to have made such studies of the silica frustule in any detail (see also Kahane, 1935; Vinogradov, 1939). He found that the diatom frustule was made of pure silicic acid in a sub-colloidal condition, giving only a diffuse pattern without any powder rings characteristic of silica. In the fossil state the diatoms have an opal-like structure. The present X-ray studies on diatom frustules have yielded results which are different from those of Rogall, and indicate that the silica present in the frustule contains crystalline α-quartz as a component.

Materials and Methods

The following materials were investigated: (1) *Isthmia nervosa* growing on red alga in pure formation, near San Francisco, California, U.S.A., which were collected during 1954 and preserved as an air dried sample, (2) A mixture of *Cyclotella meneghiniana* and *Gomphonema parvulum* (3) *Fragilaria brevistriata*. The specimens of (2) and (3) were collected from some water tubs in the Botany Department, University of Madras. (4) Fossil diatoms were obtained from deposits found at Lampoc in California, Florida in the U.S.A., and Lake Sumner and Oomaru in New Zealand.

X-ray studies were made with a ' Unicorn ' rotation camera. The radius of the cylindrical camera was 3 cm.; while the X-ray radiation used was Ni-filtered Cu Kα radiation with wavelength 1·542 Å. The diameter of the X-ray collimator used was 0·5 mm.

Suitably cleared material of *Isthmia nervosa* diatoms were also studied with a ' Norelco ' Philips microcamera, with the distance between the specimen and film equal to 1·76 cm. This distance was obtained by calibrating the camera with NaCl powder. The diameter of the X-ray collimator of the microcamera was 0·004″ and one single diatom frustule of *Isthmia nervosa* cleaned with potassium dichromate and sulphuric acid was adjusted to be in the X-ray beam.

RESULTS

1. *Isthmia nervosa.*—Untreated air-dried material (Fig. 1) as well as material preserved in rectified spirit (Fig. 2) did not give any prominent X-ray diffraction powder rings but only diffuse halos. Blackening, due to low angle scatter, up to about 8·8 Å was recorded, while two diffuse halos of spacing 4·10 Å and 2·09 Å were also present. These were similar to the halos obtained with silica gel when untreated (Fig. 3) as well as with silica gel when treated with potassium dichromate and sulphuric acid (Fig. 3 *a*). However, on repeated cleaning with potassium dichromate and sulphuric acid these diatoms gave a powder ring pattern, the most prominent rings having Bragg spacings of 3·36 Å, 2·51 Å and 1·832 Å (Fig. 5). This same material when studied in the microcamera showed prominent rings at 3·35 Å, and 4·26 Å (Fig. 6). For the complete X-ray analysis data *see* Table I.

2. *Cyclotella meneghiniana and Gomphonema parvulum mixture.*—This collection when cleaned and studied, yielded crystalline powder patterns (Figs. 7, 8 and 9) with the most prominent powder rings having ' *d* ' spacings of 3·35 Å, 4·43 Å and 1·820 Å (*see* Table I). The powder ring system in every case was due to α-quartz. The X-ray powder pattern of pure α-quartz is shown in Fig. 11 for comparison.

3. *Fragilaria brevistriata.*—These diatoms also gave a similar powder ring system with prominent ' *d* ' spacings of 3·36 Å, 4·37 Å and 1·809 Å. Here again the powder ring system is due to α-quartz. The diffraction pattern given by these diatoms is shown in Fig. 10.

4. *Fossil diatoms.*—Fossil diatoms from Florida and Lampoc in the U.S.A., and Oomaru and Lake Summer in New Zealand yielded crystalline powder diffraction patterns with the most intense rings having spacings of 3·35 Å, 4·28 Å, 1·820 Å and 4·35 Å (*see* Table I). Bragg spacings for

Table I

Bragg spacings in Å for various diatom specimens

Present-day specimens						Fossils		α-Quartz (from A.S.T.M. Index)		Pectin 'd' in Å	B.D.H. silica gel 'd' in Å	Fe₃O₄	
Cyclotella meneghiniana and Gomphonema parvulum mixtures	Fragilaria brevistriata	Isthmia nervosa — Air-dried powder specimen	Specimens after cleaning	Micro-camera results	Lampoc (in U.S.A.)	Oomaru and Florida (same values)	Lake Sumner, New Zealand	'd' in Å	Intensity			'd' in Å	Intensity
10·05 w	..	Blackening up to 8·8 Å	..	..	..	..	10·05 w	..	..	10·05 vvw	Blackening upto 8·50	..	..
..	7·36 w	..	..	..	..	..	7·03 w	..	..	7·03 m	..	..	..
..	..	..	..	..	..	..	58·1 vvw	..	..	..	..	..	..
4·43 m	4·37 m	4·10*	4·10*	4·04 w	4·28 s	4·27	4·35 s	4·26	35	4·43 s	4·05*	4·86	30
3·73 w	..	..	..	4·20 s	3·73 w	3·75	3·70 w	..	..	3·89 m	..	..	..
3·35 s	3·36 s	..	3·36	3·35 vs	3·35 vs	3·35	3·35 vs	3·343	100	3·48 w	..	..	..
..	..	..	..	3·12 w	..	..	..	..	..	3·14 m	..	2·97	60
2·91 w	2·60 w	..	2·54	..	2·44 w	2·94	2·01 vw	..	..	2·88 w	..	2·53	100
2·57 m	2·56 m	..	..	..	2·8 w	..	2·57 m	2·458	12	2·50 m	..	2·43	10
..	..	..	..	..	2·12 w	2·44	2·44 mw	2·282	12	..	..	..	..
2·29 vw	2·14 m	2·09*	2·01*	..	2·04 w	2·28	2·28 mw	2·128	9	2·16 m	2·05*	2·10	50
2·12 m	1·98 w	..	1·83 w	..	1·82 m	2·12	2·12 mw	1·980	6	2·01 w	..	..	..
1·98 w	1·81 m	..	..	..	1·66 w	1·99	2·04 mw	1·817	17	..	..	..	..
1·82 m	1·66 w	..	..	..	..	1·82	1·82 ms	1·672	7	..	..	1·714	40
1·67 m	..	..	..	..	..	1·66	1·66 m	1·659	3	..	..	1·615	60
1·54 m	1·51 w	..	1·54 w	..	1·54 w	1·54	1·54 m	1·541	15	..	..	1·484	70
1·45 w	..	..	..	..	1·47 w	1·43	1·49 w	1·453	3	..	..	..	..
..	..	..	..	..	..	..	1·43 vw	1·382	7	..	..	1·326	10
1·37 m	1·38	..	1·38 w	..	1·38 m	1·38	1·38 m	1·375	11	..	..	1·279	30
..	..	..	..	..	..	..	..	1·372	9	..	..	..	..
..	..	..	..	..	..	1·29	1·29 vw	1·288	3	..	..	..	..
..	..	..	..	..	..	..	1·247 vw	1·256	4	..	..	..	..
..	..	..	..	..	..	1·185	1·185 w	1·184	4	..	..	..	..
..	..	..	..	..	..	..	1·177 w	1·180	4	..	..	..	..

* Diffuse rings.   w : Weak.   ms : Medium strong.   s : Strong.   vvw : Vrey-very weak.   vs : Very strong.   vw : Very weak.   m : Medium.   mw : Medium weak.

α-quartz are also given. It may be seen that the results obtained from the present study are in close agreement with the values for α-quartz. The fossil X-ray diffraction patterns (Figs. 12, 13, 14 and 15) can be compared with the pattern given by pure α-quartz powder (Fig. 11).

DISCUSSION

Thus it appears that the silica in these diatoms, which are both fresh-water and fossil diatoms, is α-quartz. The only anomaly is the result obtained from the study of untreated samples of *Isthmia nervosa* which presents a problem. Is there a difference between the silica as present in living diatoms and the silica in the treated diatoms which are dead, and the fossil diatoms? One solution, that suggests itself, is that there is some amorphous material which clouds the X-ray diffraction patterns and that this is removed either by chemical clearing using potassium dichromate and sulphuric acid, or during fossilization in various degrees. This naturally leads on to another question. Is the silica purely an inorganic deposition of α-quartz, or is there an organic substance in these frustules as well? If there is such an organic substance, what is its relationship with the silica which is α-quartz?

Liebisch (1929–30) does not believe that there is an admixture of organic material and that the silica is laid purely as an external layer. Mangin (1908), [*see* also Desikachary (1956)], feels that the silicate is deposited within the substance of a pectic material. Brieger (1924) also says that the silica is deposited in the shell as an organic complex. A large number of organic silicates are now known; but none have been identified so far from the diatoms. The results obtained from the present investigations agree so well with α-quartz, that the possibility of its being some other silicate seems out of question. However, an organic substance has been reported from fossil diatoms. Hahn (1925) has reported a gel-like protein (cornuit) in Kieselguhr from the deposits of Luneburg earth. It is albuminous and contains $2 \cdot 8\%$ dry substance and $0 \cdot 07\%$ ash, chiefly a diatomaceous residue. The diffusion of dyes into the gel, the adsorption of dyes and the swelling correspond to gelatin. The cornuit sol has a higher viscosity and surface tension than H_2O. The protective action of the sol was 30 times stronger than gelatin.

(*i*) *Origin of diffuse rings in untreated present-day diatoms.*—In view of this evidence, namely that a gelatin-like protein is probably present in diatoms, the X-ray diffraction pattern of thermally denatured collagen, which is practically the same as that of gelatin, was obtained. This is shown in Fig. 4. It will be noticed that the pattern is very similar to that of silica gel,

and as will be seen from Table I, the broad maxima also correspond to the same spacings in both the cases. Thus, it is perfectly possible that there is a large amount of protein in the fresh untreated diatoms and that the X-ray pattern of the diatom (*e.g.*, Figs. 1 and 2) is essentially that of the protein. When the protein is removed by cleaning, the pattern of α-quartz shows up (Fig. 5).

(*ii*) *Occurrence of α-quartz in fresh diatoms.*—It may be mentioned that the X-ray patterns of most denatured proteins (*e.g.*, albumin, etc.) are alike and are very similar to Fig. 4. On the other hand, they are also similar to that of silica gel. However, silica gel is not readily dissolved by treatment with sulphuric acid and dichromate, nor is it converted to α-quartz by this treatment (*cf.* Figs. 3 and 3 *a*). Consequently, the α-quartz must be originally present in the diatom itself and it has been masked by the large amount of proteinous material which is present in the fresh diatoms. In the fossil diatoms on the other hand, most of the protein would have been removed, so that they give the pattern of α-quartz very clearly. It is significant to note that the rings of α-quartz given by the fossil diatoms are quite sharp and that they extend right up to the back reflection region (note in particular the rings at the extreme left and right in Figs. 13, 14 and 15). This shows that the material occurs in a well crystallised form.

(*iii*) *Probable occurrence of pectin.*—It will be readily noticed, on comparing the diffraction pattern of α-quartz (Fig. 11) with those of the fossil diatoms in Figs. 12–15, that there are a number of additional lines in the latter, particularly within the strongest line of spacing $d=3\cdot34$ Å of α-quartz. A study of these indicated that these were close to those of pectin (*see* Table I). Consequently, the diffraction pattern of a mixture of α-quartz and pectin in equal proportions was obtained and is shown in Fig. 16. The intensity distribution as well as the spacings of the diffraction rings in this pattern are seen to be in good agreement with those observed in some of the fossil patterns, particularly the well-crystallised fossil diatoms from Lake Sumner in New Zealand (Fig. 15). Thus, there seems to be evidence to show that pectin is present in the diatom frustules. The near identity of the pectin and quartz spacings seems to indicate that oriented crystallization of pectin with silica is present in the diatom.

(*iv*) *Occurrence of other components.*—In *Isthmia nervosa* diffraction rings which are diffuse and which probably belong to an organic substance were observed with a spacing of $2\cdot1$ Å. Silica gel does give such a ring faintly at $2\cdot05$ Å. On the other hand, gelatin and degraded collagen also give such a ring. Consequently, if silica gel is eliminated on the grounds that

it cannot be removed by washing with acid, there seem to be good grounds for assuming that protein (probably related to gelatin) is present as a component of the frustule. There are a few other rings which are weak in intensity and are not as yet correlated with α-quartz. The possibility that these rings may not be due to pectin, but may belong to some iron or aluminium compound was also investigated. It is possible that some of these additional rings observed may belong to Fe_3O_4. The spacings for Fe_3O_4 are also given in Table I. But they do not rule out the presence of pectin, since there are still additional lines unaccounted for by Fe_3O_4. However, no aluminium oxide or any of its modifications could be detected from the diffraction patterns.

It is probable that the proportion of the organic substance varies with the species. By the partial removal of these organic substances by alkali or acid treatment (*see* Jorgensen, 1955) the silica is rendered proportionately soluble. One does not know whether these organic substances play a role in making the frustule insoluble ordinarily. There is need for study of this problem in greater detail.
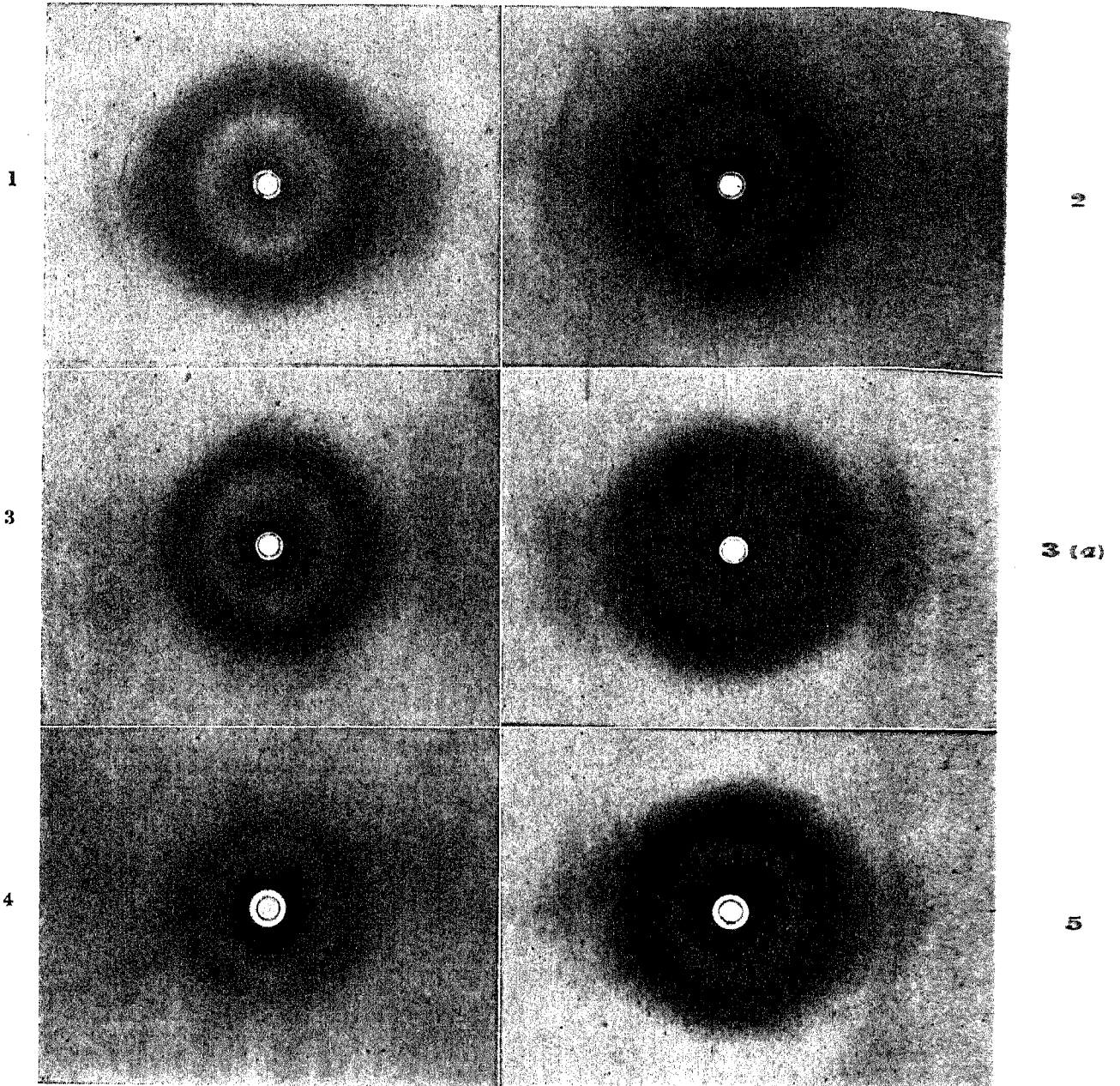
SUMMARY

This paper describes the X-ray diffraction studies carried out on a large number of present-day, as well as fossil diatom frustules. From the diffraction patterns given by the various specimens, it has been concluded that the silica present in diatom frustules is not in an amorphous or sub-colloidal state as was supposed by previous workers but that it is crystalline α-quartz. Although crystalline α-quartz is present in present-day diatoms, the degree of crystallisation is much less than in the fossilized specimens. In addition to the crystalline quartz content, there is present in all diatoms an organic component which might possibly be a protein. In the present-day diatoms this organic component occurs in considerable proportion so that its X-ray diffraction pattern tends to mask the pattern due to crystalline α-quartz. On fossilization, however, a considerable amount of the protein content is lost, while the silica content becomes more predominant; and more and more crystalline with time. Evidence for the occurrence of pectin in diatoms is also presented.

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FIGS. 1-5