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Separation of diatom valves and girdle bands from *Coscinodiscus* diatomite by settling method

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Abstract Diatom valves and girdle bands are useful micro–nano materials in nanotechnology and micro manufacturing. A settling method is used to extract high-purity diatom valves and girdle bands from *Coscinodiscus* diatomite. The average models of diatom valves and girdle bands are established. *Stokes Law* and hydrokinetic theories are used to analyze the settling velocity of valves and girdle bands. Based on the calculation results, settling experiments are carried out, by which clean diatom valves with purity of 80% and girdle bands with purity of 90% are obtained. This method can be applied to other particles separation problem to separate micro-particles with similar radius but different sectional area.

List of symbols

F_g	Equivalent gravity
F_d	Fluid drag force
μ	Fluid viscosity
g	Acceleration of gravity
S_c	Particle section area perpendicular to settling orientation
ρ_s	Density of particle
ρ_0	Density of liquid medium
k	Drag coefficient
v	Settling velocity
d	Valve diameter
d_0	Micropore diameter
h	Valve thickness
D	Girdle band diameter
R	Inradius of girdle band
H	Height of girdle band
n	Average number of micropores
r	Radius of girdle band bore
V	Particle volume

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Introduction

Diatoms provide abundant delicate structures for nanotechnology and micro manufacturing. Its frustules consist of silica valves and girdle bands and have a complex symmetry structure with an inerratic arrangement of multilevel pores in its surface [1]. Transparent and adsorptive are also excellent features of this micro glass material. In recent years, more and more research group took part in diatom study for its interesting structures and potential applications especially in nanotechnology [2, 3], such as the structural rigidity tests of diatom shells and girdle bands [4], formation investigation of diatom frustules [5], replication of porous diatom structure [6], oil-extraction from diatom [7], drug delivery with diatom frustules [8], photonic application of diatom valves [9], using diatom for solar energy [10], and so on.

Diatomite is a kind of low cost mineral which contains mass of frustules. Most of frustules in diatomite have separated into valves and girdle bands. The diatom valves, possessing bigger specific surface area than girdle bands, are the contributing parts in diatomite absorbent and filter [11] and are broadly investigated for nanotechnology application, such as mask-pattern in lithographic application [12], micro-optic device, structure replication [6], adsorption of gold particles [13], and self-assembly connection to glass slide [14]. The girdle bands, transparent circular material with good elastic deformation property [4], possess photonic crystal properties [15–17] and also have great potential applications in micro manufacturing, such as micro seal ring fabrication. Supposing the valves and girdle bands could be extracted from diatomite separately, two kinds of useful micro parts will be obtained.

Stokes law is usually used to analyze the separating process of micro spherical particles with different radius. Although both valves and girdle bands are not spherical particles and they have similar outer diameter, yet in this article it was proved that stokes settling method could be used to separate these two kinds of particles, with some hydrokinetic theories' help.

Methods

Derivation of settling velocity of non-spherical particles

Stokes law is preferable for analyzing the settling characteristics of spherical particles, yet it could not be directly applied to separate valves from girdle bands, which exhibit different three-dimensional structures and both are not spherical. Some investigation indicated that objects with same volume but different shapes have different surface contact stress in water, such as ellipsoidal shell and spherical shell [18], so we turned to hydrokinetic theories and derivate settling velocity of non-spherical particles. The equivalent gravity of particle immerged in liquid medium is written as

$$F_g = (\rho_s - \rho_0)g \cdot V. \qquad (1)$$

Here, F_g is the equivalent gravity, ρ_s the density of particles, ρ_0 the density of liquid medium, g the acceleration of gravity, and V the volume of particle. According to the flow resistance formula, the fluid resistance of particles in micron scale is proportional to fluid viscosity, square root of the particle sectional area perpendicular to settling orientation, and particle settling velocity. Therefore, the fluid drag force on the particles can be expressed as:

$$F_d = k\mu\sqrt{S_c} \cdot v, \qquad (2)$$

where k is the drag coefficient. The equivalent gravity and fluid drag force reach balance when a particle reaches its maximum falling velocity, that is

$$F_g = F_d. \qquad (3)$$

Combining Eqs. 1–3, we obtain the settling velocity

$$v = \frac{(\rho_s - \rho_0)g \cdot V}{k\mu\sqrt{S_c}}. \qquad (4)$$

If valves and girdle bands settle with different velocity, then settling method could be applied to separate these two kinds of particles.

Modeling and calculation of diatom valves and girdle bands

The structure of the *Coscinodiscus* Ehrenberg diatom valve is shown in Fig. 1a; it looks like a semi-culture dish with micropores in its surface. In order to simplify the calculation, we presented a valve model as a wafer with uniform micropores (Fig. 1b). The water viscosity at room temperature is $\mu = 0.8937 \times 10^{-3}$ Pa s and the diatom true density is $\rho_s = 2.1 \times 10^3$ kg m^3. The parameters of the valves take the following values: the valve diameter $d = 40$ µm, the valve thickness $h = 3$ µm, the micropore diameter $d_0 = 0.4$ µm, the micropores number $n = 640$, the fluid drag coefficient $k = 10.63$ (calculated with *Stokes settlement law* in situation of 10–100 µm spherical particles).

The volume of valve is

$$V = \left(\pi\frac{d^2}{4} - n \cdot \pi\frac{d_0^2}{4}\right) \cdot h = 3.528 \times 10^3 \ \mu m^3$$

The area of the largest cross sections is

$$S_{max} = \pi\frac{d^2}{4} - n \cdot \pi\frac{d_0^2}{4} = 1.176 \times 10^3 \ \mu m^2$$

The area of the smallest cross sections is

$$S_{min} = d \cdot h = 120 \ \mu m^2$$

Then the settling velocity of valves is calculated by Eq. 4:

$$v_{min} = \frac{(\rho_s - \rho_0)g \cdot V}{k\mu\sqrt{S_{max}}} = 1.18 \times 10^{-4} \ m/s,$$

$$v_{max} = \frac{(\rho_s - \rho_0)g \cdot V}{k\mu\sqrt{S_{min}}} = 3.34 \times 10^{-4} \ m/s,$$

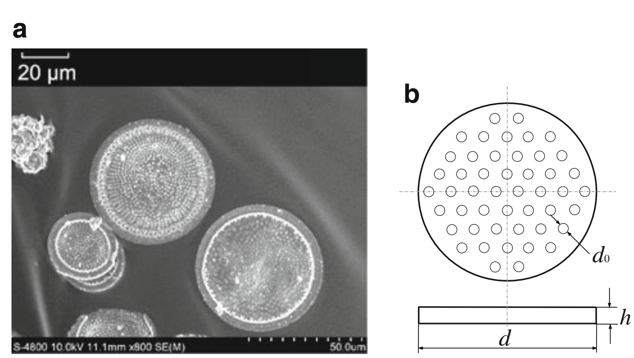

Fig. 1 Diatom valves SEM photographs (**a**) and its approximate model (**b**). The scale bar in **a** is 20 µm

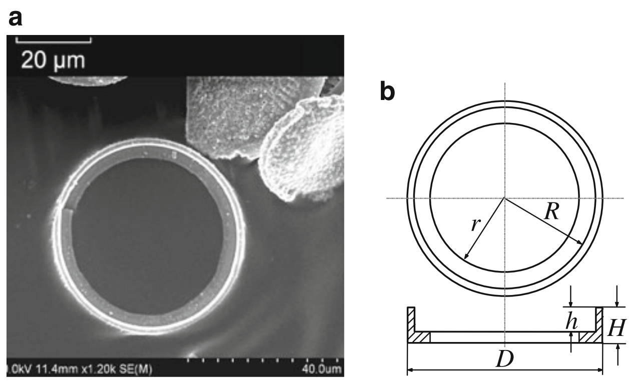

Fig. 2 Girdle bands SEM photographs (**a**) and its approximate model (**b**). The scale bar in **a** is 20 μm

That is, the settling velocity of valves is variable in range (1.18, 3.34) × 10^{-4} m/s.

Figure 2a displays the girdle bands of diatom *Coscinodiscus*, which was simplified to a ring model shown in Fig. 2b. The size parameters of an average girdle band take the following values: $D = 44$ μm, $R = 21$ μm, $r = 18$ μm, $H = 4$ μm, $h = 3$ μm, other parameters are as above.

Then the settling velocity of girdle bands is calculated with the same method:

$$v_h \in (4.22, 5.04) \times 10^{-5}\ \text{m/s}.$$

From the calculation, we can see that the difference between the settling velocity of valves and that of girdle bands. In static water, diatom valves will take 4.8–13.8 min falling 10 cm depth, and girdle bands need 36 min in contrast, which means settling method may be effective to separate girdle bands from valves.

Materials and experimental

Materials and purification

The diatomite is from Linjiang Sailite Diatomite Co. Ltd. The frustules in diatomite are mostly belong to *Coscinodiscus* Ehrenberg. Compared with frustules derived from living diatom cells by chemical treatment or baking, the proportion of undivided frustules (Fig. 3a) in diatomite is <30%, and the majority particles are separate valves (Fig. 3b) and girdle bands (Fig. 3c). One reason may be the frustules has been buried too long that the linkage systems between valves and girdle bands were damaged [19].

Diatomite contains not only complete diatom particles but also a large number of micro-organism debris and impurities stained in frustules surface, so purification should be the first step to get rid of debris to obtain clean diatom valves and girdle bands. The methods are as follows: the diatomite is mixed with anhydrous ethanol at a mass ratio of 1:10 in beaker. After ultrasonic cleaning for 30 min at room temperature, the mixture is filtrated with filter cloth with 20 μm pores (rinse the mixture on filter cloth with deionized water simultaneity). The residue on filter cloth is placed in a clean beaker filled with deionized water.

The residue is mostly clean diatom valves and girdle bands (Fig. 4), which will be used for settling experiment later. The proportion of undivided frustules reduces from nearly 30% to <10%, one possible reason is some frustules were separated during the ultrasonic cleaning process.

Settling velocity observation

In order to verify the calculation results in "Methods" section and determine the optimal suspension duration, we designed a settling experiment to observe the settling characteristic of diatom valves and girdle bands. The concrete process is as follows.

Fifty milliliter cleaned mixture is poured into 50-mL graduated cylinder. The cylinder is fully stirred and then put still for 5 min. Five milliliter pipettes are used to divide the mixture in the cylinder into 10 small beakers sequentially from top to bottom. A sample of 10-μL mixture is taken from each small beaker, and the particles such as valves and girdle bands in the samples are counted separately via a microscope (Fig. 5a is a scheme of the

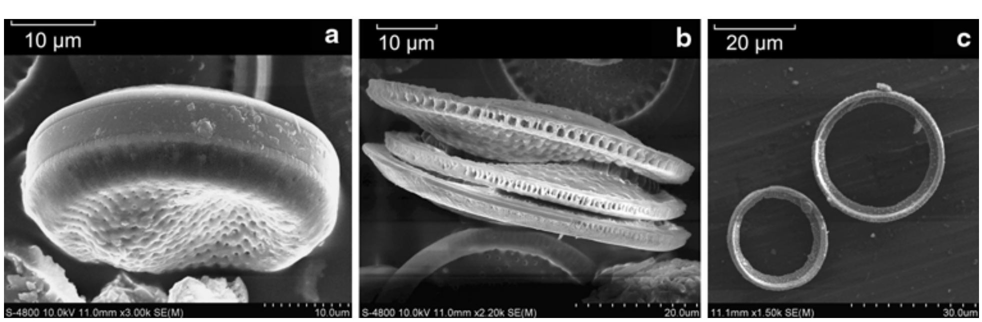

Fig. 3 Main particles in diatomite: undivided frustules (**a**), diatom valves (**b**), and girdle bands (**c**). The scale bars in **a**, **b**, and **c** are 10, 10, and 20 μm, respectively

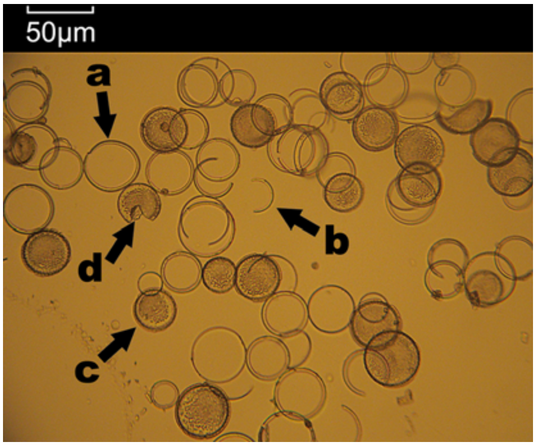

Fig. 4 Diatom frustules after purification. The scale bars is 50 μm. The *arrows* in figure indicate four different particles, arrows *a* and *b* are girdle band and incomplete girdle, arrows *c* and *d* are valve and incomplete valve

operations above). The process was carried out for three times, and the average was taken to reduce error. After the sample data processed in Matlab, the particles distribution curve for 5 min is drawn. The particles distribution curves for 10, 15, and 20 min are also achieved in same method with the duration of settling changed to 10, 15, or 20 min.

Settling separation of diatom valves and girdle bands

Based on the results of optimal settling duration in "Results of settling velocity observation and discussion" section, the experimental method of separating diatom valves and girdle bands from mixture are designed as follows. Figure 5b is a flow chart of the experiment.

Step 1: Fifty milliliter cleaned mixture is taken and filled into a 50-mL graduated cylinder. The cylinder with mixture is fully stirred and then put still for 20 min. The mixture above 15-mL scale is transferred into a clean beaker A by pipette.

Step 2: The cylinder is filled to 50-mL scale with deionized water again and then put still for 20 min. After that, the mixture above 15-mL scale is transferred into the same beaker A again.

Step 3: After repeating step 2 two times, the mixture in graduated cylinder and beaker A is filtrated with filter cloth with 20 μm pores, the residue on each filter cloth is rinsed to clean beakers B and C with deionized water separately.

Results and discussion

Results of settling velocity observation and discussion

As the results of experiment in "Materials and purification" section, The particle distribution curves for different settling time are shown in Fig. 6. According to the statistical results, particles in the cleaned mixture consist of 28.6% diatom valves, 41.2% girdle bands, 3.5% incomplete valves, and 26.7% incomplete girdle bands. Figure 6 shows how the four kinds of particles settle in 20 min in still water. Ninety percent diatom valves fell below 15-mL scale after 20 min, when only 41% girdle bands and 38% incomplete girdle bands settled below this scale. The subsequent observation shows that 20% girdle bands and 28% incomplete girdle bands still suspend above 15-mL scale 1 h later.

Comparing the experimental results with the calculated values in "Methods" section, we find that the calculated settling velocity of diatom valves is in accordance with the actual movements, but the girdle bands settle with a speed slower than calculated value. One possible reason is the girdle bands are so small in volume that they are more

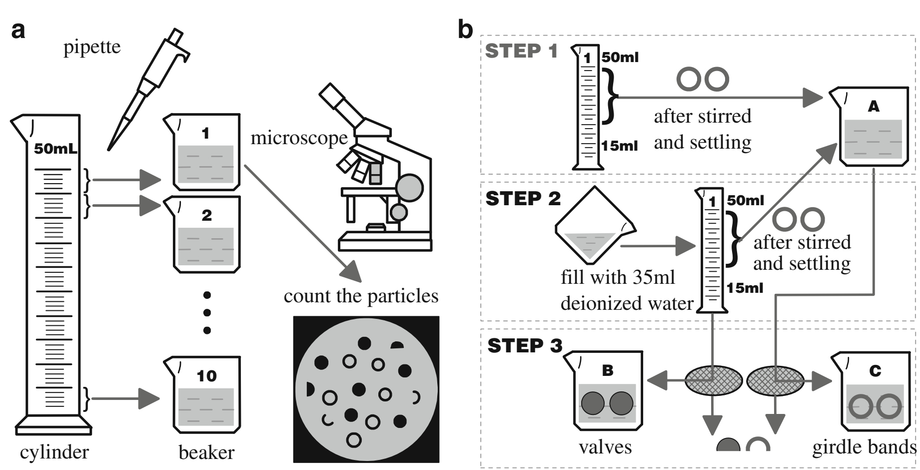

Fig. 5 Schematic illustration of particles sampling of each liquid layer (**a**), flow chart of settling separation of diatom valves and girdle bands (**b**)

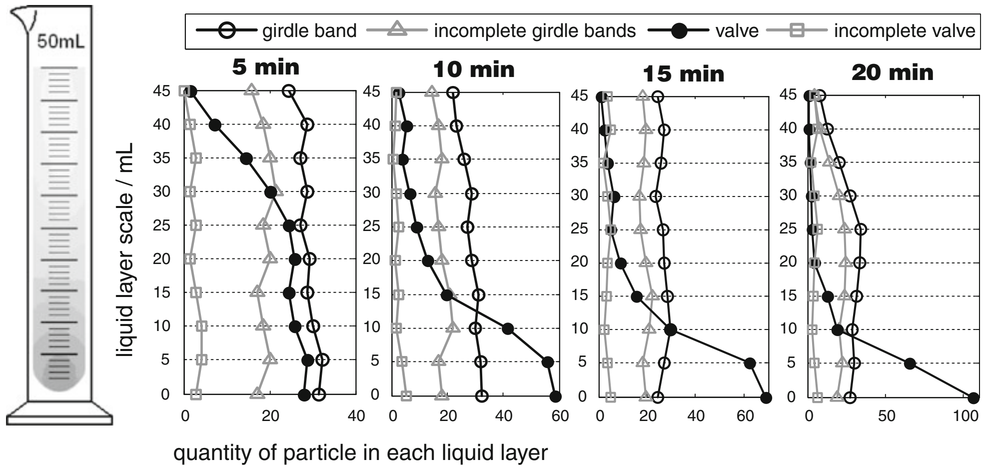

Fig. 6 Particles distribution curves for 5/10/15/20 min. The *dots* in curves denote the quantity of the particle in each liquid layer

affected by Brownian motion than bigger particles, which slows down the settling movement.

The tests above verified the difference of settling velocity of valves and girdle bands; also implied 20 min is the suitable suspension duration for settling separation.

We also tried other settling methods, such as pouring the particles mixture from top of the water and then putting the graduated cylinder still. However, the results indicated that the method is not convenient for separating valves and girdle bands. Even with a 5-L beaker and low concentration of frustule mixture, more than 30% girdle bands settled to 20 cm deep bottom of beaker at the beginning (Fig. 7), the valves and girdle bands could not distribute in different fluid layers as they did in "Settling velocity observation" section. Therefore, stirring and putting still are more effective methods.

Results of separation experiment and discussion

Figure 8 shows the results of experiment in "Settling velocity observation" section. Figure 8a and b shows the particles in beakers B and C separately. The most particles

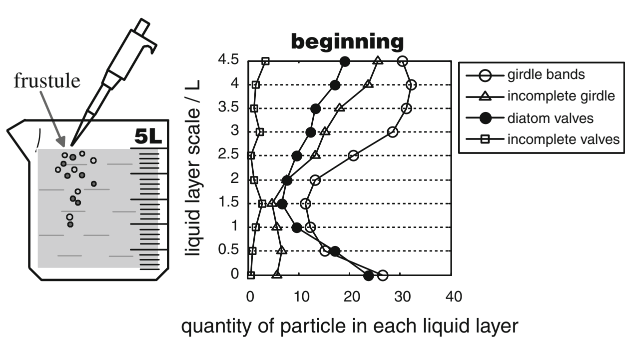

Fig. 7 Particles distribution curves in the beginning. The *dots* in curves denote the quantity of this particle in each liquid layer

in beaker B are diatom valves, with a proportion of nearly 80% (Fig. 8a), the other particles are girdle bands and fragments. Other experiments illustrate that repetition of step 2 in "Settling velocity observation" section could reduce the proportion of girdle bands in beaker B. The particles in beaker C are 90% girdle bands and a few fragments (Fig. 8b). Figure 8c and d shows some details of separated valves and girdle bands. From Fig. 8c, we can see that the micropores and brims of the valves are scatheless after settlement, and from Fig. 8d we can notice that each complete girdle bands possess a joint.

Conclusions

In this article, a settling method is applied to separate valves and girdle bands, respectively, from *Coscinodiscus* diatomite. The modeling of the valves and girdle bands were built and the theoretical analysis was done, and series of experiments was carried out which successfully verified the feasibility of the method. Finally, the high-purity valves and girdle bands were achieved separately. This study is valuable and can be applied in many fields. First, this settling method offers two kinds of high-purity frustule components for the study of diatom nanotechnology. Second, this method could be regarded as a processing technique of diatomite material, for elimination of girdle bands may improve the efficiency of diatomite product. Third, this method will offer the researchers a new way to process diatom and do the structural observation [20]. Finally, it proved that particles with similar size but different shapes could be separated by settling method, and the settling method may be applied to other similar particles separation problem, such as other frustule parts, silica bodies (phytoliths) [21], or other micro-particles.

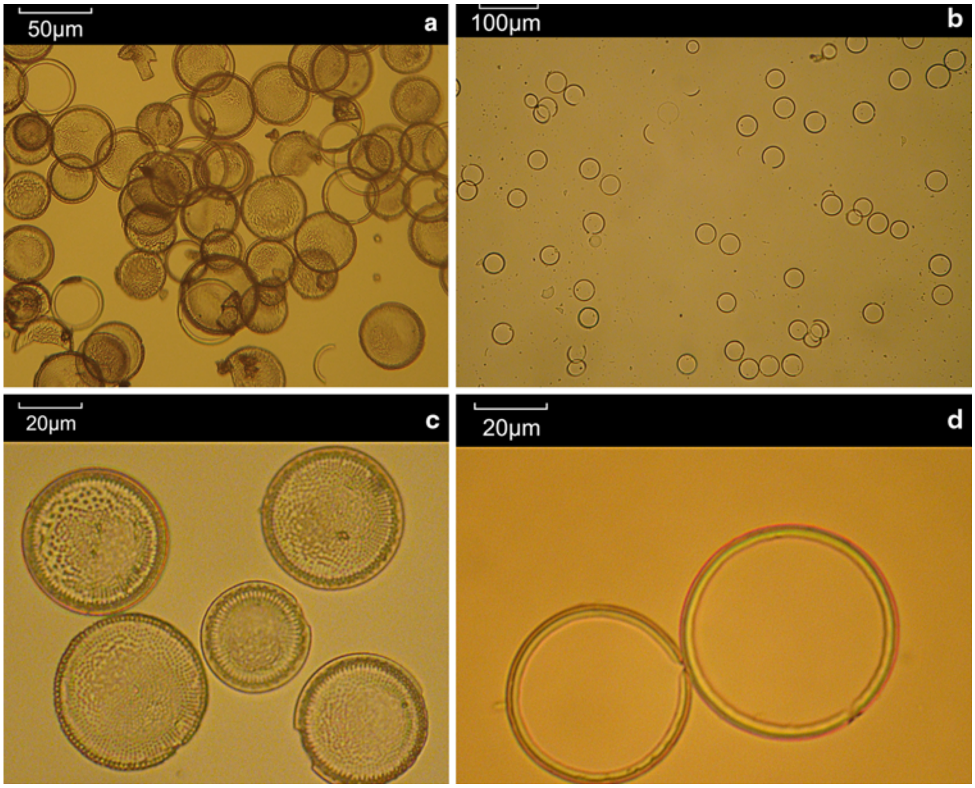

Fig. 8 Microscopic images of diatom valves (**a**, **c**) and girdle bands (**b**, **d**) extracted from diatomite. The scale bars in **a**, **b**, and **c/d** are 50, 100, and 20 μm, respectively

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