

Water quality test based on dielectrophoretic
measurements of fresh water algae *Selenastrum*
capricornutum

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Summary

A method is described whereby dielectrophoresis of algal cells is used to perform rapid water quality analysis, specifically detecting the presence of CuSO_4 . The dielectric collection spectrum of the fresh water alga *Selenastrum capricornutum* was determined for a range of concentrations of CuSO_4 between 25 mg L^{-1} to 0.25 mg L^{-1} for exposure times of 15 minutes and 18 hours. In all cases increasing the concentration of CuSO_4 reduced cell collection, but a step reduction was observed in collection between 2 mg L^{-1} and 5 mg L^{-1} . This method has potential for forming a rapid, low-cost test for water quality with broad specificity and significantly reduced analysis time compared to current methods.

Contents entry

An electrostatic technique is demonstrated for assessing CuSO_4 effects on algae after 15 minutes exposure to the compound in solution.

1. Introduction

About 80 % of all diseases and over one third of deaths in developing countries are caused by the consumption of contaminated water¹. The majority of these waterborne diseases are caused by bacteriological or other biological contamination in the water. Nevertheless, a significant number of health problems occur as a result of chemical contamination. In order to ensure that water supplies are drinkable, the water quality has to be monitored. Since it is not possible to measure water for every toxin, global markers have to be used, which test the general toxicity. Recent tests measure the growth inhibition of microorganisms such as algae or bacteria. The European standard water quality test² compares the growth rate of fresh water algae that are cultured in the test substance to the growth rate of those that are cultured in a control substance under defined conditions. The cell density is measured every 24 h during a minimum period of 72 h.

Natural water contains lots of impurities, especially minerals. The concentrations of other material ranges from a few milligrams per litre to about 35 g L⁻¹ in seawater³. Some contaminants come from erosion of natural rock formations. Others are discharged from factories, farmlands or used by customers in their homes and gardens. Pollution from these activities may enter the ground water or may be deposited from the atmosphere. Most of the minerals and man-made chemicals that are found in water are harmless, but at a certain concentration these contaminants make the consumption of the water unsafe. The contaminants are divided onto two groups according to whether they cause acute or chronic health effects. Acute effects occur within hours or days after consuming contaminated water. To this group belong bacteria, viruses and some chemicals. Chronic effects occur after people consume a contaminant at high levels for many years. To this group belong mainly chemicals, such as disinfectants, solvents and pesticides. For this reason governments of all countries have set standards for the quality

of drinking water. In the United States of America the Environmental Protection Agency (EPA) sets drinking water standards. One of these standards is the Maximum Contaminant Level (MCL), which is the highest level of a contaminant that is allowed in drinking water. The Maximum Contaminant Level Goal (MCLG) is the level of a contaminant in drinking water, below which there is no known or expected risk to health⁴.

The technique used in this study is based on dielectrophoresis. Dielectrophoresis is the motion of particles caused by dielectric polarisation effects in non-uniform electric fields. The dielectrophoretic properties, such as permittivity and conductivity of both the membrane and cytoplasm of cells change after death. These differences in the dielectrophoretic properties and hence in the structure of the membrane of the cells, can be measured. In an earlier study Markx *et al.*⁵ used dielectrophoresis to separate viable and non-viable yeast cells. In this paper we demonstrate a new method for water quality tests, which is based on the ability of AC-electrokinetics to distinguish live cells from dead cells rapidly. It is shown that by using this method after centrifugation of the cells, the time that is needed to measure the toxicity of cells can be reduced to 15 minutes of exposure to copper sulphate solutions. In this study. The technique is applied to characterise algae cells of the same type that is used in the European standard water quality test².

1.1 Water quality tests

In order to measure the water quality, indicators are needed for the concentration of many different chemicals, minerals and microbes. Former tests were based on macroscopic organisms such as rats, fish or daphnia. Fish can tolerate a certain amount of pollution without any serious effects. In water with low concentrations of nutrient material, many types of organisms will be present, whereas in streams with high concentrations of nutrients, bacteria predominate. These conditions are unsuitable for animals

and higher plants³. A balanced ecosystem indicates a good water quality. Another method is the 10-day chronic toxicity test using *Daphnia magna* or *Daphnia pulex*. Larval daphnids are placed in separated vessels and exposed to different concentrations of a test media over a 10-day period. Concentrations are measured every day and mortality, reproduction and growth are recorded. These results are compared with standard samples⁶. Recent tests use microorganisms such as algae and bacteria for the determination of toxic effects of chemical compounds. The European standard test for water quality is based on growth inhibition of fresh water algae. Monospecific algae strains are cultured for several generations in a medium consisting of a range of concentrations of the test substance, nutrient concentrate, water, test substance and exponentially growing algae cells. During a minimum period of 72 h the cell density of the test solution has to be measured at least every 24 h. The growth inhibition is measured as a reduction in growth or growth rate relative to control cultures which are grown under identical conditions². In this study we will deal with the algae *Selenastrum capricornutum*. Algae growth is affected by a number of physical and chemical factors, e.g. temperature, light intensity pH and nutrient concentration. Any change in these factors has effect on the growth rate of the algae. Contaminants have very different effects on the algae. Heavy metals are the most common used toxic agents. Many algae tolerate concentrations near 1 ppm Copper sulfate, but are susceptible to higher concentrations. Copper, cadmium, mercury, lead and zinc damage the structure of cytoplasts and hence cause reduction of photosynthesis. Copper ions also inhibit the synthesis of proteins. Nitrogen and phosphorus disturb algae growth and reproduction. Heavy metals stimulate this uptake of nutrients.

1.2 Dielectrophoresis

If a particle is suspended in an electric field, it polarises and establishes a dipole. The direction and magnitude of the induced dipole depends on the frequency, the magnitude of the electric field and on the dielectric properties of the cell and the medium^{7,8}. If the particle suspended in a medium that is less polarizable than it, the particle moves towards the region of highest electric field. This is true even if the field changes direction, as happens when an AC electric field is applied between the electrodes. This

phenomenon is called positive dielectrophoresis. If the particle suspended in a medium more polarizable than it, the electric field will be distorted around the particle. It moves away from the high field towards the region of lowest electric field. This motion is called negative dielectrophoresis. Particles experiencing positive dielectrophoresis are attracted to electrode edges; those experiencing negative dielectrophoresis are repelled from them.

The polarizability of the particle and medium depends on the frequency of the applied electric field. Depending on the frequency, a particle can experience either positive or negative dielectrophoresis. The cause for this behaviour is that the orientation of the acquired dipole depends on the accumulation of charge on either side of the interface between the particle and medium. The amount of the accumulated charge depends on the impedance of the particle and the medium and on the frequency of the electric field. At a defined frequency window, the net behaviour of the system changes from being dominated by the particle to being dominated by the medium. The particle goes from being attracted (positive dielectrophoresis) to being repelled by the electrodes (negative dielectrophoresis)⁸.

A uniform electric field causes the formation of a dipole within the material of a polarizable particle and an accumulation of charges on its surface. Because the charges are equal and opposite, the Coulomb forces cancel out. There is no net force on the particle. If the particle is suspended in a non-uniform field the magnitude of the charges and therefore of the Coulomb forces on each side are different. This is called the dielectrophoretic force.

The dielectrophoretic force F_{DEP} , acting on a spherical body is given by⁸:

$$\mathbf{F}_{\text{DEP}} = 2 \pi r^3 \varepsilon_m \text{Re} [K(\omega)] \nabla E^2 \quad (1)$$

Where r is the particle radius, ε_m is the permittivity of the suspending medium, ∇E^2 is the gradient of the strength of the applied electric field squared and $\text{Re} [K(\omega)]$ is the real part of the Clausius-Mossotti factor given by¹¹:

$$K(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + \varepsilon_m^*} \quad (2)$$

Where ε_m^* and ε_p^* are the complex permittivities of the medium and the particle, respectively. ε^* is defined as¹¹:

$$\varepsilon^* = \varepsilon - \frac{j\sigma}{\omega} \quad (3)$$

Where ε is permittivity, j is the square root of -1 , σ is the conductivity and ω is the angular frequency of the applied electric field. The Clausius-Mossotti factor is dependent on the frequency and can take either positive or negative values, which depends on whether the cell is more or less polarizable than the medium. If $\text{Re} [K(\omega)]$ is positive, the particle experiences positive dielectrophoresis. It moves to regions of highest field strength and is attracted by the electrodes. The number of collected particles is

proportional to the magnitude of $K(\omega)$ and hence the force. If $\text{Re}[K(\omega)]$ is negative, the particle experiences negative dielectrophoresis and is therefore repelled by the electrodes.

2 Material and Methods

2.1 Cell preparation

Green algae *Selenastrum capricornutum* (CCAP 278/4) were grown in *Euglena Gracilis* Medium. (2g L⁻¹ Yeast extract (Oxoid L21), 2g L⁻¹ Tryptone (Oxoid L42), 1g L⁻¹ Sodium acetate trihydrate, 1 g L⁻¹ Lab-Lemco powder (Oxoid L29, 10mgL⁻¹ CaCl₂). This solution was autoclaved at 121°C and 1 bar overpressure for 15 min and 10 ml of the algae solution were added. The algae were grown at 20°C under 6 hours of white light per day. For experimentation, algae cells were centrifuged (Centra-3C, International Equipment Company, England) at approximately 180x g for 10 minutes. The pellets were washed and resuspended in distilled water. A cell density of approximately 10⁶ cells per ml, counted by using a haemocytometer (Improved Neubauer, Weber England, Depth 0.1 mm, 1/400 mm²), was used for dielectrophoretic measurements.

Cells were then separated into 900µL aliquots, and were centrifuged (Labnet Force 16-centrifuge) at approx. 1300x g for 10 min. The pellets were suspended in CuSO₄-solutions with concentration between 0.2mg L⁻¹ and 25mg L⁻¹ and different incubation times (15 minutes and 18 hours); in addition a control sample was centrifuged but resuspended in distilled water. After exposure to CuSO₄, the cell solutions (including the control sample) were centrifuged (Labnet Force 16-centrifuge) at approx. 1300x g for 10 min. and the pellets were washed and resuspended in distilled water. These solutions were then analysed using dielectrophoresis.

2.2 Dielectrophoretic measurements

To build the dielectrophoresis chamber two needles with a sharpened edge to maintain cell collection in the focal plane [9, 10] were placed 60 μm apart from each other, and connected with a capacitor and a signal generator in a series circuit. The capacitor blocked the DC offset and the signal generator generated the electric field. For each experiment 180 μL of the cell solution were pipetted into the dielectrophoresis chamber and a cover slip was placed over the chamber, in order to reduce the drifting effect of the cells during the measurement. To carry out dielectrophoresis, a 20 V peak-to-peak sinusoidal signal was applied to the electrode array, which was generated by a signal generator (Thurbey Thandar instruments, TG120, 20 MHz Function Generator). Different frequencies of the range of 100 kHz-20 MHz were applied. The cell movement was viewed under a light microscope (Zeiss) with a video camera, television and with a video images were recorded for further analysis. The cell collection was recorded after 30 s for each frequency.

2.3 Trypan Blue Test

0.5 ml of 0.4 % Trypan Blue solution and the same amount of algae solution were transferred to a test tube and mixed thoroughly. This solution was allowed to stand for 10 min. Live and dead cells took up the dye, but live ones metabolised it into transparent products. The solution was viewed under a light microscope (Zeiss) and live and dead cells were counted.

3. Results and Discussion

Following the determination of the collection rate, the Clausius-Mossotti factor was fitted to the collection spectra was modelled using the multi-shell model¹¹; this method has been applied to yeast cells¹², viruses¹³, cancer cells⁹ and drug-resistant and drug-sensitive bacteria¹⁰, latex beads¹⁴. Since

crescent-shaped bodies were used for this study, the results do not provide a true reflection of the actual dielectric values, but by determination of the effective dielectric values it is possible to gain insight into the effects of CuSO_4 on algal cells.

At least three experiments were performed for each characterisation with the overall pattern remaining the same. Figure 1 shows the representative collection rate of live algae cells measured for 30 s in distilled water with a measured conductivity of 0.2 mS m^{-1} . The cells exhibited at all tested frequencies positive dielectrophoresis, which decreased monotonically across the frequency range. The model was fitted to collection data by varying the value of the conductivity and permittivity of cytoplasm, membrane and cell wall. The dielectrophoretic parameters were then determined from the best-fitting curve. The best-fit for the data for untreated cells is shown in fig. 1. The modelling was performed using the following parameters: mean cell radii of inside of membrane ($2.09 \times 10^{-6} \text{ m}$), outside of membrane ($2.11 \times 10^{-6} \text{ m}$) and inside of cell wall ($2.36 \times 10^{-6} \text{ m}$), obtained from the literature¹⁵. These parameters remained constant during the curve fitting. The best fit provided the following *effective* parameters; cell wall relative permittivity 30 and conductivity 20 mS m^{-1} ; membrane conductivity 200 mS m^{-1} ; cytoplasmic conductivity 10 mS m^{-1} and relative permittivity 160. Note that these are *effective* parameters and hence do not necessarily reflect the *actual* values.

The algae were treated with CuSO_4 , which has an effect on the structure of the chloroplast. After treatment and centrifugation, there was an evident reduction in the number of cells visible. The remaining cells generally showed the same dielectrophoretic behaviour as the live cells. The Trypan Blue test showed that the visible cells were alive and hence not affected by the CuSO_4 ; modelling the data showed that the effective dielectric properties of these cells were the same as those of the untreated cells. However, some cells were observed exhibiting negative dielectrophoresis, which was not

observed in the untreated cells and indicating that a population of the algae had changed in their dielectric properties significantly enough to prevent them collecting at the electrode edges.

This effect was found to vary significantly with the concentration of CuSO_4 used. Figure 2 shows the average number of cells collected as a function of concentration of CuSO_4 . In each case exposure was for 15 minutes and cells were centrifuged before analysis. The collection rate depended on the cell density of the measured solution. During the CuSO_4 -treatment, the cell density decreased. It was possible to view this decrease in the cell density by dielectrophoresis. The higher the concentration of the CuSO_4 -solution, the higher was the decrease of the cell count for each applied frequency. Significantly, a distinct step-change in the number of cells counted was visible between 2mg L^{-1} and 5mg L^{-1} CuSO_4 , where the cell count reduces by a factor of approximately x3 across the whole spectrum. This reduction remains for higher concentrations of CuSO_4 . Why the cell density decreases is not clear yet. It is not the CuSO_4 alone that destroys the algae cells; the cell density stays constant during the treatment with CuSO_4 , but after centrifugation the cell density decreases significantly. It is known that CuSO_4 destroys the structure of the chloroplast in algae cells¹⁶. This may weaken the cells, causing them to disintegrate during centrifugation.

Studies were also performed on the collection rates of live algae cells and cells treated with CuSO_4 -solution for 18 h under the same conditions as the cells which were treated with CuSO_4 for 15 min. The numbers of the collected cells after a treatment of 15 min. were significantly lower than the numbers of collected cells after a treatment for 12 h, which more closely resembled the cell numbers of the control samples. The dielectrophoretic collection spectra for cells after 12 hours exposure are shown in figure 3. As can be seen, the step reduction in collection between samples treated with 2mg L^{-1} and 5mg L^{-1} CuSO_4 is still evident, though the magnitude of reduction in cell count is not as significant. It is known

that *Selenastrum capricornutum* can become resistant against CuSO_4 ¹⁷. We suggest that a small proportion of the cells exposed to CuSO_4 survived the treatment (a hypothesis in keeping with the small number of cells exhibiting unchanged behaviour after 15-minute exposure) and reproducing to pre-treatment numbers.

4. Conclusion

The study demonstrated the success of employing dielectrophoresis to determine the effects of CuSO_4 on algal cells. Whilst treated cells in CuSO_4 solutions behave similarly to untreated cells, after centrifugation a significant reduction in collection is observed at concentrations greater than 2mg L^{-1} . CuSO_4 is orally toxic. Acute oral LD50 (male rat) is 472 mg kg^{-1} . CuSO_4 is toxic also to fish and aquatic organisms. The fish toxicity critical concentration is 235 mg L^{-1} and plant toxicity is 25 mg L^{-1} ¹⁸. For those critical concentrations this test is sensitive enough. This system could therefore form the basis for a new water quality test. Such a system offers many advantages over current tests involving the measurement of turbidity of algal solutions after 72 hours exposure. The method demonstrated here reduces the time needed for an accurate and repeatable determination on algal survival significantly from 72 h to 15 min.

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FIGURE CAPTIONS

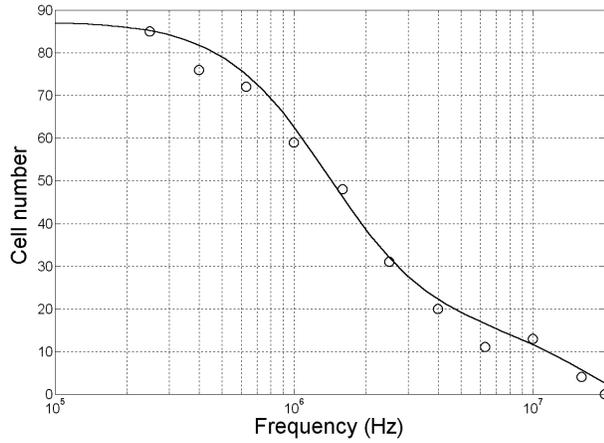


Figure 1. Dielectrophoretic collection spectrum of *Selenastrum capricornutum* in 2mSm^{-1} solution. The data points represent the dielectrophoretic collection numbers, whilst the curved line represents the best fit to the data using a multi-shell dielectric model.

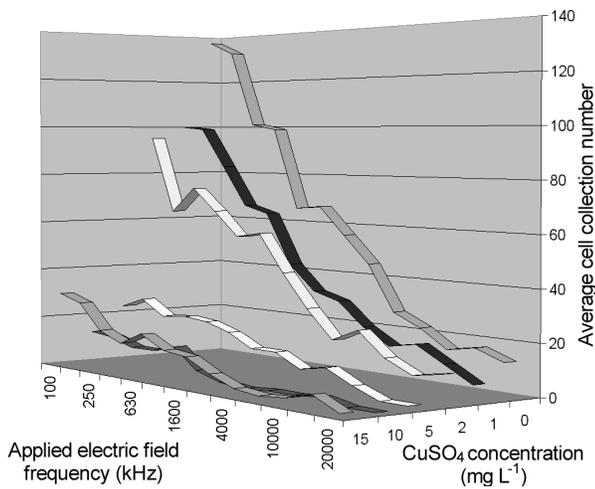


Figure 2. Dielectrophoretic collection spectra of *Selenastrum capricornutum* in 2mSm^{-1} solution, after 15 minutes suspended in CuSO_4 solutions of various concentrations (indicated). Note the visible step-change in cell collection between 2mg L^{-1} and 5mg L^{-1} .

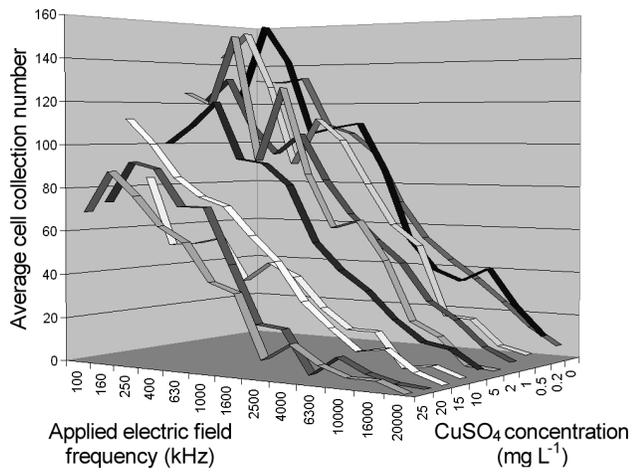


Figure 3. Dielectrophoretic collection spectra of *Selenastrum capricornutum* in 2mSm^{-1} solution, after 12 hours suspended in CuSO_4 solutions of various concentrations (indicated). Note the visible step-change in cell collection between 2mg L^{-1} and 5mg L^{-1} .