Label-free analysis of water-polluting parasite by electrochemical impedance spectroscopy

T. Houssin a,b,*, J. Follet a,c, A. Follet a,d, E. Dei-Cas a,e, V. Seneza a,b

a Univ Lille Nord de France, F-59000 Lille, France
b Institut d'Électronique, de Microélectronique et de Nanotechnologie, CNRS, UMR 8520, France
c Laboratoire de Biotechnologie des Micro-organismes, Institut Supérieur d'Agriculture, EA 3609, France
d Univ Lille, Labo E&S, F-59000 Lille, France
e Laboratoire d'Ecologie du Parasitisme, Institut Pasteur de Lille, EA 3605, France

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A new, label-free, real time and non-invasive method is presented to detect the presence of infectious parasites in water and determine accurately their concentration by electrochemical impedance spectroscopy (E.I.S.) using interdigitated microelectrode array. Cryptosporidium parvum was taken as model. Buffer influence on parasite detection was investigated by comparing parasites suspended in purified water versus phosphate buffer saline. It was shown that a low conductive buffer is required for parasite detection. Different suspensions of C. parvum oocysts were measured in purified water. By fitting resulting electrochemical impedance spectrums with an equivalent electrical circuit, solution conductance was extracted. Conductance increased linearly with C. parvum oocyst concentration. The reasons of conductance modification induced by parasite presence are discussed. Cell constant was calculated for circular interdigitated electrode arrays. Thus sample conductivity can be obtained from raw impedance spectrums and it was established that water conductivity was proportional to C. parvum oocyst amount. This relationship can be expressed by: $\sigma = 2.88228 \times 10^{-6} \times C[\text{oocysts/µL}] + 1.64565 \times 10^{-4}$ with $R^2 = 0.99$. In this way, E.I.S. can be used as a rapid alternative to current parasite counting procedures which consists in fluorescent staining and microscopic observation. The distinction between dead and living parasites by E.I.S. was also approached. Between 10 kHz and 100 kHz, electrochemical impedance showed a difference of 15% between dead and living oocysts.

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1. Introduction

In 2006, United Nations report reminded 1.1 billion people still do not have access to safe drinking water and 2.6 billion does not have adequate sanitation causing global health issue with dramatic infant mortality. These figures correspond respectively to 17% and 40% of the world population. Parasite contamination represents one of the main obstacle to water disinfection and a serious threat to public health in developing countries (United Nations, 2006). Even in developed countries, periodic parasite outbreaks demonstrate that current methods for water treatment still require some improvements (Karanis et al., 2007). For instance, an epidemics of cryptosporidiosis in Milwaukee (Wisconsin, USA) involved more than 400 000 persons and caused 69 deaths (Mac Kenzie et al., 1994). Cryptosporidium parvum is one of these waterborne parasites causing cryptosporidiosis in humans and animals, a severe diarrhoea whose main symptoms are violent stomach cramps, nausea, vomiting, diarrhoea, dehydration, fever and weight loss (Center for Disease Control, 2009). In persons with weakened immune systems like HIV people or young children, cryptosporidiosis may be deadly (Petersen, 1992). Furthermore, no effective specific treatment against cryptosporidiosis is available. C. parvum is a protozoan, unicellular parasite. The infectious life stage of C. parvum is oocyst, a thick walled 5 µm diameter structure, which allows C. parvum to contaminate water and survive for years without investing any hosts. Once it is ingested, it delivers into the intestine four sporozoites which are able to infect intestinal epithelial cells causing then diarrhoea. Usually the ingestion of dozens of oocysts is needed to provoke cryptosporidiosis (Dupont et al., 1995) but the disease can be induced in humans by a few C. parvum oocysts of highly virulent strains (Okhuysen et al., 1999).

Current method mainly applied for C. parvum detection is based on immunomagnetic separation (IMS), which consists of fixing anti-C. parvum antibody on paramagnetic beads. Antibody is able to react with C. parvum antigen present at the surface of oocysts to finally capture them with a magnet. To determine then oocyst concentration, they need to be previously labelled by a fluorescent
antibody and subsequently counted using a fluorescent microscope (U.S. EPA 1622 Method, 2001a,b). All these different steps are labour intensive and time consuming. *C. parvum* detection is not sufficient: assessment of oocyst viability is needed for a complete diagnosis. Most common methods used for viability assessing are sporozoite excystation and vital dye exclusion (Guyot et al., 2001). Infectious power can be tested on neonatal conventional or adult immunodepressed mice (Certat et al., 2007) or on cell culture (Slifox et al., 1997). These approaches require cumbersome infrastructures and skilful techniques. Therefore, *C. parvum* detection and viability assessment would definitively gain by employing real time, automatically and faster techniques offered by biomedical engineering. It becomes necessary to develop new tools for low-cost and easily practicable analysis, all the more for an issue affecting particularly developing countries.

Alternative methods using biomems integrating optical (Kang et al., 2008), mechanical (Campbell and Mutharasan, 2007) or microfluidic (Taguchi et al., 2007) transducers showed interesting results concerning *C. parvum* detection without any application straightforwardly implementable. Concerning electrical method, Goater used electrorotation to assess *Cryptosporidium* viability (Goater et al., 1997). Regrettably, this method still requires microscopic observation making its use and integration difficult.

Besides, electrochemical impedance spectroscopy (E.I.S.) is a technique generating more and more interest from biologists and pharmaceutical industry. This method presents several advantages: real-time detection, label-free analysis, non-invasive sensing, easiness of integration and high-throughput screening (Spegel et al., 2008). Main drawback is the difficulty to implement specific detection. Indeed, electrochemical detection implies that any chemical reaction may produce conduction modification. Schwan was one of the first to propose a complete model to understand dielectric dispersion of biological cells suspended in electrolyte (Bothwell and Schwan, 1956). Giaever cultivated adherent cells on microelectrodes and showed cell migration can be monitored by EIS (Giaever and Reese, 1993). Since these precursory studies, many researchers have been led in the use of E.I.S. for biological applications like glucose monitoring for diabetic patients (Caduff et al., 2006), capacitive cytometry (Sohn et al., 2000), drug analysis (Leung et al., 2005), viral infection study (Campbell et al., 2007) and the analysis of cellular morphology changes (Arndt et al., 2004). Since the last decade, E.I.S.-based systems have been commercialized by companies like ACEA bioscience (Yu et al., 2006), Cellkey (Verdonk et al., 2006) and Applied Biophysics (Keese et al., 2004) and are getting more and more employed in pharmacological and biological laboratories.

In this paper, we show a new method to detect and quantify parasites water contamination by E.I.S. using interdigitated micro-electrodes. The purpose of this label-free method is to use E.I.S. as a replacement for current fluorescent staining and manual counting. Parasite capture and concentration were not assessed here but are still required in the proposed protocol. Buffer effect is studied with an equivalent electrochemical circuit. Oocysts suspended in purified water with different concentrations are discriminated by complex impedance spectrums. Conduction is extracted by curve fittings. Cell constant is calculated and linear reallization between sample conductivity and *C. parvum* oocyst concentration is identified. Finally, *C. parvum* oocyst viability assessing is approached.

2. Materials and methods

2.1. Materials

The following materials and chemicals are used to carry out experiments: square Pyrex substrates (5 cm × 5 cm × 0.7 mm thick) (Verre Industrie, Marne La Vallée, France); acetone and isopropanol alcohol (Carlo Erba Réactifs, Val de Reuil, France); Texpure™ Cleanroom Cleaning Solution (Conformat, La Garenne Colombes, France); Photosensitive resin AZnLof2020, developer AZ 326 and Remover PG™ (MicroChem Corporation, Newton, MA, USA); PolyDiMethylSyloxane (PDMS) (Arrow Electronique, Villeneuve d’ascq, France); Phosphate Buffer Saline (PBS) (Amresco, Baltimore, MD, USA); Water for injection (WFI) (Fresenius, Sèvres, France); SubMiniature version A (SMA) connectors, printed circuit board (PCB) (Radiospares, Beauvais, France); Oocysts of “Iowa” strain of *C. parvum* (the “Iowa” strain was originally isolated by H. Moon from a symptomatic calf in Ames, IA, USA) and FL-Crypt-a-Glo™ Kit (Waterborne Incorporation, New Orleans, LA, USA); Trypsin and taurocholic acid (Sigma, Saint Louis, MO, USA).

2.2. Biomems fabrication and mounting

Four sensors are fabricated on a Pyrex substrate (Fig. 1A). Each sensor consists in a 8 mm circular arrays of interdigitated fingers (width: 4 μm, spacing: 4 μm, Fig. 1B). After substrates cleaning with Texpure™, acetone, isopropanol and dehydratation, titan adhesive layer and gold are deposited on substrates and patterned by optical lithography, evaporation and lift-off process (Fig. 1C). These steps define sensors geometry and electronic contacts layout (Fig. 1D).

Four circular wells (diameter: 8 mm) are punched in a PDMS block. This PDMS piece is aligned with the four electrodes arrays and non permanently bonded to substrate by capillary forces. The resulting biochip is finally mounted on a home-made PCB and interconnections between gold pads on the biochip and copper leads on the PCB are achieved by copper wires bonding (Fig. 1A).

2.3. Oocysts preparation

*C. parvum* oocysts were stored at 4°C in PBS supplemented with Penicillin, Gentamicin, Amphoterin B and 0.01% Tween 20. Before using, they were washed by centrifugation at room temperature for 15 min at 1000 × g, and resuspended in PBS, pH 7.4 or WFI. The period of time between the delivery of oocysts in our laboratory and their use in experiments was always shorter than two months. From a pellet of *C. parvum* oocysts resuspended in WFI, a sample concentrated to 2 × 10⁶ oocysts/ml was obtained. Serial dilutions in WFI were made in order to have oocyst suspension in the concentration range from 2 × 10⁶ to 1 × 10⁴ oocysts/ml. Each diluted sample concentration was controlled on a Malassez hemacytometer. For observation (Fig. 1E), oocysts were labelled with an anti- *Cryptosporidium* monoclonal antibody (FL-Crypt-a-Glo Kit) and viewed by epifluorescence under UV excitation through an FITC wavelength filter on a Nikon 80i microscope at ×630 magnification. Pictures were captured with a Nikon DXM 1200C digital camera.

2.4. Oocyst inactivation treatment

After centrifugation steps, pelleted oocysts were suspended in WFI. Samples were diluted in order to obtain a final concentration of 1 × 10⁶ oocysts/ml and aliquotted. According to a previous developed protocol (Fujino et al., 2002), each aliquot was submitted to heat inactivation treatment for 1 min at 70°C followed by a step at 0°C for 1 min. Loss of viability was controlled by in vitro excystation assay (Black et al., 1986) and the count of 500 events corresponding to intact or empty oocysts. Excystation percentage was calculated using the equation (number of empty oocysts/total oocysts, intact and empty) × 100. Samples exhibiting viability rate
lower than 2% were considered as representative of a dead oocyst population.

2.5. Electrochemical impedance measurement

Impedance measurements were performed with an impedance analyzer Agilent 4294A connected to the biochip through 1 m long coaxial cables. A homemade LabVIEW® program drives automatically impedance analyzer measurements through an IEEE GPIB interface. The 4294A measure both absolute value of impedance and phase shift on a broad frequency range (here 100 Hz to 1 MHz). In order to not damage oocysts, ac input signal was 10 mV. The recorded spectrum was made of 201 measurement points and was obtained within 45 s. In the measurement procedure, 110 μl of solvent, either PBS or WFI, were first dispensed in the 4 wells and covered with parafilm to prevent liquid evaporation. After 10 min, to allow a perfect solvation of microelectrodes, electrochemical impedance of buffer was recorded. Then the wells were emptied and refilled with 110 μl of C. parvum suspension. After waiting 5 min to stabilize mass transport of buffer and oocysts, electrochemical impedance of oocyst suspension was recorded for at least 6 min. Prior to new concentration analysis, wells were rinsed by pipetting several times 200 μl of WFI. This cleaning step mechanically removes C. parvum oocysts adhering on microelectrodes and ensured to recover initial electrochemical impedance value obtained with buffer solution. In this way, electrochemical impedance measurements were made in identical conditions for every C. parvum oocyst suspension sample. All experiments were performed at room temperature, at least in triplicate. Thus standard deviations plotted in figures were calculated from at least three measurements.

3. Results and discussion

3.1. Buffer influence

It has been shown that electrochemical impedance of biological cells is frequency dependent (Foster and Schwan, 1989). Because of insulating properties of their plasma membrane, their electrochemical impedance tends to be capacitive at low frequencies (kHz) and conductive in medium frequencies (MHz). To measure the influence of buffer conductivity on parasites electrochemical impedance, we considered two buffers, a low conductive one: WFI and a highly conductive one: PBS. Measurements of their conductivity with a conductivity meter LF-96 (WTW, Weilheim, Germany) give the values of 14.12 mS/cm (14.12 × 10⁻¹ S/m) (pH 7.3) for PBS

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**Fig. 1.** (A) Picture of the biochip bonded to the PCB. (B) SEM picture of electrodes on array edge. (C) Side view schematic of biochip structure. (D) Schematic of electrodes layout on biochip. Schematic in inset explains the method to calculate cell constant. (E) Fluorescent and DIC picture of C. parvum oocysts on microelectrodes. Oocysts were stained with a green fluorescent marker. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)
Fig. 2. Bode plots of the electrochemical impedance for C. parvum oocysts suspended at a concentration of 1000 oocyst/μl in WFI (A and C) and PBS (B and D). Equivalent electrical fits are obtained with the equivalent electrical circuit of Fig. 3.

and 1.65 μS/cm (1.65 × 10^{−4} S/m) for WFI. C. parvum oocysts were suspended in 110 μl of either WFI or PBS at a concentration of 1000 cells/μl. Fig. 2 shows Bode plots of electrochemical impedances for (a) WFI buffer with or without oocysts (Fig. 2A and C) and (b) PBS buffer with or without oocysts (Fig. 2B and D).

The electrical circuit represented in Fig. 3 is a first order behavioural model of the electrochemical impedance system. This type of circuit has been previously used to analyse experimental electrochemical impedance spectrums of biological cells (Ehret et al., 1996). \( R_{\text{interconnect}} \) represents the resistive component caused by interconnections, \( C_{\text{dielec}} \) models sample dielectric properties, \( R_{\text{sample}} \) stands for sample conductive properties and liquid–electrode interface is modelled as \( Z_{\text{interface}} \). This interface corresponds to a double layer structure which cannot be represented by ideal components such as capacitor and resistor. This element is called a constant phase element (Grimnes and Martinsen, 2000) whose phase is frequency independent and different than \(-90^\circ\). It can be defined by:

\[
Z_{\text{interface}} = \frac{1}{K(j\omega)^n}
\]  

where \( j^2 = -1 \), \( \omega \) is the angular frequency of the applied voltage in Hz, \( K \) is in Ss\(^n\) (siemens × second\(^n\)) and \( n \) is a real number with \( 0 < n < 1 \). Our electrode system is based on a 2-point measurement so \( R_{\text{interconnect}} \) and \( Z_{\text{interface}} \) of the two electrodes are in series and have been replaced by a unique equivalent component. The calibration of parameters \( (R_{\text{interconnect}}, C_{\text{dielec}}, R_{\text{sample}}, Z_{\text{interface}}) \) appearing in equivalent electrical circuit is performed using non linear square fitting to experimental data with ORIGIN 7.5 software (Integral Software, Paris, France).
impedance phase spectrums. Equivalent electrical fits are obtained with the equivalent electrical circuit of Fig. 3. Overall electrochemical impedance was dominated by capacitive effects (\(Z_{\text{interface}}\)) induced by the interfacial double layer localized at microelectrodes surfaces. At high frequencies (>500 kHz), the overall electrochemical impedance of the system was dominated by dielectric properties of water (\(C_{\text{dielec}}\)). At intermediate frequencies (1 kHz to 500 kHz), the overall electrochemical impedance depended mainly on suspension conductivity (\(R_{\text{solution}}\)). Fit of experimental spectrums for WFI buffer without C. parvum oocysts yields: \(C_{\text{dielec}} = 1.48 \times 10^{-8}\) F, \(K = 1.9338 \times 10^{-6\pm 7.671 \times 10^{-5}}\) \(\text{S}\), \(n = 0.89879\) \(\pm 7.5 \times 10^{-4}\) and \(R_{\text{sample}} = 853.46829 \pm 1.64243\) \(\Omega\). A good agreement on the entire spectrum for this unique set of parameters was observed in Fig. 2A and C.

For experiment with PBS, Bode plot showed a capacitive behaviour up to 300 kHz. Since PBS is very conductive, influence of electrical double layer on the overall electrochemical impedance and its consequent cut-off frequency were shifted towards higher frequencies compared to WFI results. Above 300 kHz, the influence of PBS conductivity is observable. The positive phase observed above 1 MHz was due to parasitic self effects caused by coaxial cables. Fit of experimental spectrum for PBS buffer without C. parvum oocysts yields: \(C_{\text{dielec}} = 3.1964 \times 10^{-12\pm 3.3118 \times 10^{-15}}\) F, \(K = 3.9089 \times 10^{-6\pm 2.1167 \times 10^{-9}}\) \(\text{S}\), \(n = 0.92664\) \(\pm 1.1 \times 10^{-4}\) and \(R_{\text{sample}} = 1.18949 \pm 0.07772\) \(\Omega\). K was more important for PBS than for WFI. Indeed, since PBS is more conductive than WFI, it presents more ionic charges at the electrode–electrolyte interface. Again, since PBS presents high conductivity, \(R_{\text{sample}}\) is much smaller than for WFI. The value obtained for \(C_{\text{dielec}}\) shows the equivalent circuit for PBS buffer can be reduced to a resistance in series with a CPE element.

### 3.1.1. Suspensions without parasites

For both WFI and PBS, calibrated value of \(R_{\text{interconnect}}\) remains very low: 1.2 \(\Omega\). Concerning WFI, at low frequencies (<1 kHz), the overall electrochemical impedance was dominated by capacitive effects (\(Z_{\text{interface}}\)) induced by the interfacial double layer localized at microelectrodes surfaces. At high frequencies (>500 kHz), the overall electrochemical impedance of the system was dominated by dielectric properties of water (\(C_{\text{dielec}}\)). At intermediate frequencies (1 kHz to 500 kHz), the overall electrochemical impedance depended mainly on suspension conductivity (\(R_{\text{solution}}\)). Fit of experimental spectrums for WFI buffer without C. parvum oocysts yields: \(C_{\text{dielec}} = 1.48 \times 10^{-8}\) F, \(K = 1.9338 \times 10^{-6\pm 7.671 \times 10^{-5}}\) \(\text{S}\), \(n = 0.89879\) \(\pm 7.5 \times 10^{-4}\) and \(R_{\text{sample}} = 853.46829 \pm 1.64243\) \(\Omega\). A large decrease of \(R_{\text{sample}}\) compared to WFI buffer without oocysts was observed, while \(C_{\text{dielec}}\) was unchanged and K was slightly modified. In conclusion, oocysts only modify conductive properties of WFI buffer. Hypo-osmotic conditions experienced by C. parvum oocysts in WFI may explain conductivity change. Indeed, electrical properties of C. parvum structure were not yet determined but Dalton et al. (2001) had previously shown that Giardia intestinalis, another intestinal protozoan parasite with close physiological structure, had a highly conductor internal fluid (0.8 ± 0.25 m⁻¹). Moreover Park et al. (1997) had already shown that G. intestinalis releases inorganic ions like K⁺ and Cl⁻ when suspended in hypo-osmotic conditions. Although there is no report about conductivity of C. parvum oocyst internal fluid and its osmotic regulation, apparent similarities between C. parvum and G. intestinalis physiological structures suggest conduction changes may be attributed to C. parvum.

### 3.1.2. Parasite suspensions

Electrochemical impedance measurements presented in Fig. 2 showed that C. parvum oocysts had no effect on PBS electrochemical impedance. On the contrary, they had a great impact on the electrochemical impedance of WFI buffer. Applying fitting procedure to the data corresponding to WFI buffer with oocysts yields: \(C_{\text{dielec}} = 1.48 \times 10^{-8}\) F, \(K = 1.90 \times 10^{-6\pm 2.8194 \times 10^{-9}}\) \(\text{S}\), \(n = 0.892\) \(\pm 0.00029\); \(R_{\text{sample}} = 206.5523 \pm 0.61434\) \(\Omega\). A large decrease of \(R_{\text{sample}}\) compared to WFI buffer without oocysts was observed, while \(C_{\text{dielec}}\) was unchanged and K was slightly modified. In conclusion, oocysts only modify conductive properties of WFI buffer. Hypo-osmotic conditions experienced by C. parvum oocysts in WFI may explain conductivity change. Indeed, electrical properties of C. parvum structure were not yet determined but Dalton et al. (2001) had previously shown that Giardia intestinalis, another intestinal protozoan parasite with close physiological structure, had a highly conductor internal fluid (0.8 ± 0.25 m⁻¹). Moreover Park et al. (1997) had already shown that G. intestinalis releases inorganic ions like K⁺ and Cl⁻ when suspended in hypo-osmotic conditions. Although there is no report about conductivity of C. parvum oocyst internal fluid and its osmotic regulation, apparent similarities between C. parvum and G. intestinalis physiological structures suggest conduction changes may be attributed to C. parvum.

**Fig. 4.** Bode plots of the electrochemical impedance for C. parvum oocysts suspended in WFI with different concentrations. (A) Impedance magnitude spectrums and (B) impedance phase spectrums. Equivalent electrical fits are obtained with the equivalent electrical circuit of Fig. 3.

**Fig. 5.** Sample conductance versus C. parvum oocysts concentration (left axis) and sample conductivity versus C. parvum oocysts concentration (right axis).
Cryptosporidium oocyst osmotic shock and its sequent release of ions. Similar effects have been reported by Yang (2008) for bacteria suspension. The fact that PBS is usually isosmotic with eukaryotic cells, could explain why C. parvum oocysts did not release ions and no impedance change was recorded. Therefore, analysis of C. parvum oocysts through their electrochemical impedance requires a low conductive media.

3.2. Detection and accurate determination of oocyst concentration

After showing that Cryptosporidium oocysts can be detected by E.I.S, the efficiency of this method for quantifying parasite concentration was approached. Fig. 4 shows electrochemical impedance magnitude (Fig. 4A) and phase (Fig. 4B) spectrums for 10–2000 oocysts/µl suspended in WFI. As parasite concentration increased, impedance magnitude got lower and phase spectrum shifted towards higher frequency. Firstly, detection limit is lower than 10 cells/µl. Secondly, the calibration of equivalent circuit parameters shows that parasite concentration increase only changed buffer conductivity, dielectric properties remaining unchanged. Indeed, $R_{\text{sample}}$ decreased from 1290 ($\pm$3.19395) $\Omega$ for WFI sample without oocysts to 33.6 ($\pm$0.17168) $\Omega$ for WFI sample with 2000 oocysts/µl while $K$, $n$ and $C_{\text{dielec}}$ vary slightly with oocysts concentration (average $K=1.73316 \times 10^{-6}$ ($\pm$1.02104 $\times 10^{-7}$) S cm$^{-2}$ and average $n=0.89881$ ($\pm$0.01322), $C_{\text{dielec}}=1.48 \times 10^{-8}$ F). This analysis suggests once again conductance variation is more informative than raw impedance modification to standardize C. parvum concentration. Impedance changes seemed therefore rather dependent on ion release from C. parvum oocysts in water. Variations of water conductance between these experiments may be attributed to the fact that conductivity of WFI may quickly drop due to its high purity and slight contaminations (Linderholm and Renaud, 2005).

Fitting parameters can be used to determine electrical properties of parasite sample. $R_{\text{sample}}$ is representative of sample conductive properties. Sample conductance ($G_{\text{sample}}=1/R_{\text{sample}}$) was represented in function of C. parvum oocyst concentration in Fig. 5, left axis. Thus, sample conductance evolves in a perfect linear way with increasing oocyst concentration. Linear regression equation of sample conductance is given by $G(S)/C=1.43433 \times 10^{-5} \times C+7.545921 \times 10^{-4}$ where $C$ is the C. parvum oocyst concentration (oocysts/µl) and $R^2=0.99$. This relation shows that ions release from C. parvum oocysts is proportional to the number of oocysts suspended in water.

3.3. Determination of water conductivity modification induced by C. parvum oocysts

Sample conductance is directly proportional to its conductivity. This relation can be written as:

$$\sigma = \kappa G$$

where $\kappa$ is called the cell constant (geometrical factor), $\sigma$ is sample conductivity and $G$ its conductance. Cell constant depends only on geometric considerations (sample configuration, electrodes design and contact points). For planar interdigitated electrodes with constant length, cell constant can be written as (Olthuis et al., 1995):

$$k = \frac{1}{(n-1)l} \frac{2k[\cos(\pi/2) \times (w/(s+w))]^2}{K[\sqrt{1-\cos(\pi/2) \times (w/(s+w))]^2}}$$

where $N$ is the number of electrodes, $L$ the electrode length, $K$ an elliptic integral of the first kind and $w$ is the electrode width and $s$ the interelectrode space.

This elliptic integral does not have analytical solutions but Hilberg showed it can be approximated by a factor depending on geometric parameters (Hilberg, 1969; Linderholm et al., 2004). We also modified the first factor depending on electrode length to adapt it to a circular array of electrodes. Details of these calculations are reported in Appendix A.

Finally, cell constant of circular interdigitated electrodes array becomes:

Using Eq. (4), cell constant of electrodes array was calculated and a value of 0.2028 m$^{-1}$ was determined. According to Eq. (2) and using conductances obtained in Fig. 5, we calculate a conductivity for WFI equal to 1.57 $\mu$S/cm. This value is very close to conductimeter measurements (1.65 $\mu$S/cm), the error being only 4.8%. This cell constant was used to calculate the conductivity of C. parvum oocysts suspensions with various concentrations from their conductance. Fig. 5 (right axis) represents sample conductivity versus C. parvum oocyst concentration. Since conductance and conductivity are proportional, linearity with parasites concentration is conserved. Linear regression equation of sample conductivity is given by:

$$\sigma = 1.64565 \times 10^{-4} + 2.88228 \times 10^{-6} \times C$$

where $C$ is the C. parvum oocyst concentration (oocysts/µl) and $R^2=0.99$. One can observe that Y-intercept of this curve corresponds to WFI conductivity.

3.4. Viability assessment

Detection of C. parvum oocysts is not sufficient to determine water contamination. A dead oocyst will not be an infectious parasite. A total water diagnosis requires viability assessment. It is important to distinguish here viability and infectivity. Indeed, it is possible that living parasites do not contaminate their host. Even parasites apparently dead can reveal infectious to mice (Neumann et al., 2000). Nevertheless, viability gives a good approximation of infectivity. For the moment, current method used to determine oocyst viability is oocyst in vitro extracystation. It requires incubation of
oocysts in acid reactants (in order to mimic gastric microenvironment) to excyst sporozoites from oocysts and accurate counting under microscope with high magnification. Infectivity is also assessed by using cell culture or animal assay to estimate parasite growth.

As both procedures are tedious and time consuming, electrical methods could be proved to be pertinent and more efficient. Fig. 6 shows electrochemical impedance magnitude spectrums of dead and living Cryptosporidium oocysts suspended in WFI (110 µl). Both suspension concentrations are 1000 oocysts/µl.

At low (<10 kHz) and high (100 kHz) frequency, impedance magnitude values are close with overlapping standard deviations. Between 10 kHz and 100 kHz, dead Cryptosporidium oocysts have an impedance magnitude 15% lower than living ones with distinct standard deviations. According to explanations given in Section 3.2, this indicates that dead oocysts release more ions than living ones. Jenkins et al. (1997) have demonstrated that inactivated C. parvum oocysts have an increased permeability. Dye permeability assay uses this physical feature of dead oocysts as an indicator of their viability and infectivity. Higher permeability increases the ion flow from the oocyst to the external fluid (WFI), which may explain why dead oocysts present a greater impedance decrease. Viability tests were performed on C. parvum oocyst suspensions with viabilities of 0% and 100% that do not correspond to actual oocyst populations where dead and alive oocysts are randomly mixed.

4. Conclusion

The current procedure for Cryptosporidium oocyst detection in water includes basically filtration (Feng et al., 2003), concentration and fluorescence microscopic counting. The method here developed, based on electrochemical impedance measurement, was able to assess rapidly Cryptosporidium oocyst concentration in a microvolume of a low conductive fluid. The linear relationship observed between the ion release from Cryptosporidium parvum and fluorescence microscopic counting. The method here developed allows replicating easily the counts on a same sample in order to obtain complete results with statistical analysis. E.I.S. assay could be integrated in the US EPA 1622 method as a more rapid counting strategy, making it easier to perform and more reproducible.

In addition, E.I.S. and its data analysis are easily automatable thanks to the use of electronic. Partial automation would thus limit human intervention and error in the analysis. Moreover, it allows replicating easily the counts on a same sample in order to complete results with statistical analysis.

Another interesting perspective of this work is the development of a complete point-of-use water analysis system by integrating on the same biochip EIS biosensors for counting with preceding filtration, purification and concentration bioMEMS.

Finally, since the conductivity modifications reported here are caused by the ion release from C. parvum oocysts under hypoxic conditions, this detection method might be applied to other waterborne parasites having a cyst life stage, like G. intestinalis or Entamoeba histolytica.

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Appendix A. Supplementary data


References