MICRO-ELECTRICAL IMPEDANCE SPECTROSCOPY FOR PARTICLE DETECTION

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ABSTRACT

Microfluidic devices with integrated electrical detection will enable fast, low-cost or portable sensing and processing of biological and chemical samples. As an inherent property of microfabrication, micro-electrical impedance spectroscopy detectors can take advantage of AC electrokinetics for particle manipulation, leading to enhanced sensitivity. Preliminary experiments on particle detection were carried out using microelectrode pairs, and impedance spectra are compared with respect to opposite effects of dielectrophoresis and electrode polarization. The values of cell equivalent circuit are extracted for electrode optimization.

INTRODUCTION

Recent years have witnessed many developments in microfluidic and micro-total analysis systems (µTAS) through applying microfabrication techniques to biological and chemical analysis. Integrating microscale detectors with fluid transport systems on one chip has led to significant advantages, including increased resolution, faster response, smaller sample sizes, and increased parallelism of analysis.

These systems have great potentials in the fields of biotechnology, medicine development, food science, etc., where they are used to study various chemical and biological particles and to handle, separate and monitor bio- and chemical processes. Thus, particle detection is critical for improvements in micro-analysis systems.

There are mainly two groups of detection schemes for fluidic systems. One is optical detection, which is commonly realized through UV detectors, fluorescence detectors and refractive index detectors. For large-scale laboratories with sophisticated equipment and trained personnel, these detection techniques provide high sensitivity. However, the implementations and operations of these techniques are not well suited for integration with Microsystems.

Another group of detection strategies uses electrical characterization, which scans the detection volume with an electrical frequency sweep over a range of frequencies. One method is electrical impedance spectroscopy (EIS), inspecting both real and imaginary parts of impedances typically from tens of hertz to mega hertz. EIS systems are simpler to construct than optical systems, more compatible with microtechnology, as well as more reliable [1]. There have been reports on microfabricated EIS prototypes intended for microfluidic systems, which are based on changes in solution conductivity [2, 3, 4].

However, for particle detection, micro- EIS systems present another advantage, that is, utilizing dielectrophoresis (DEP) to concentrate particles for enhanced sensitivity. Particles suspended in fluid could experience forces when subjected to AC electric fields, either attracted or repelled from the electrodes according to their dielectric properties. Due to proximity of microfabricated electrodes, sufficient electric field strength can be obtained with relatively low voltages applied over the electrodes. Recent experiments have demonstrated the use of DEP for manipulation and characterization of particles, and the separation of mixtures, such as cells, bacteria, and latex spheres [5, 6, 7, 8]. Since
AC scanning signals are adopted in EIS detection, DEP can be readily combined with EIS. In some cases, the trapped bioparticles are even perforated by the electrodes using a ramped voltage to further enhance the EIS sensitivity [9]. There is a general belief that the DEP phenomenon has yet to be fully exploited for attracting target bioparticles to biosensors, thus enhancing the sensitivity of the sensor. The experiments in our work show that DEP is not species sensitive and hence species-sensitivity remains a major task of the sensor — DEP only removes the transport limitation to the sensor.

In addition, earlier work in our laboratory has shown that besides DEP, there are repulsive interactions between particles and electrode polarization [10] that may sometimes oppose the trapping effects of positive DEP. So here, we study the effects of electrode polarization and DEP on EIS for the detection of latex particles. Increased sensitivity has been obtained from aluminum microelectrodes and the impedance spectra indicate positive DEP of latent particles in the detection. However, with a more polarizable Au electrode, the repulsive interaction completely removes the enhanced sensitivity due to DEP. Particle detection by EIS with micro-electrodes must hence be designed carefully, with the myriad of electrokinetic phenomena in mind.

EXPERIMENTS

a. Microelectrode fabrication

The detection of particles was carried out with a pair of parallel microelectrodes. The electrodes were 20 mm long, 300 µm wide and 0.1 µm thick with a 20 µm separation. Planar microfabrication techniques were used to fabricate electrodes on glass substrates. The procedure is as follows: Depositing a layer of SiNₓ on glass substrates to promote photoresist adhesion, applying photoresists and patterning electrodes for metal lift-off, electron-beam evaporating 0.1 µm thick metals and immersing samples in acetone to obtain electrodes.

Two types of metals were used: Al (100 nm) and Ti/Au (10nm/90nm). For Ti/Au electrodes, Ti is the adhesion layer between the substrate and Au, and Au is in contact with electrolytes. The purpose of adopting two electrode metals is to investigate the effects of electrode polarization on electrical detection of particles. The standard potentials of Al/Al³⁺ and Au/Au³⁺⁺ half-cells are -1.66 and +1.55, respectively [11], therefore, the difference in impedance spectra from the two types of electrodes will indicate the impacts of electrode reactivity.

b. Measurements

The electrode chambers were formed by sealing silicone microchambers (PC8R-0.5, Grace Bio-Labs, Inc.) over the glass slides, so approximately 9 mm length of electrode pairs was exposed to electrolytes, and the electrode chambers have a height of 500 µm. De-Ionized (DI) water was used as testing solution both with and without particles. Polystyrene spheres at a diameter of 5 µm (Fluka Chemica 79633) were suspended in DI water at a particle concentration of ~1.0x10⁸ particles/ml as the particle solution.

The electrode impedances were measured with an Agilent 4294A impedance analyzer from 40 Hz to 5 MHz at an open oscillation level of 1.0 V rms. At this excitation level, electric field strength on the order of 10⁴ V/m is generated between two close edges of the electrode pairs.

THEORY

The impedance between the electrodes is determined by physical properties of the electrodes and the electrolyte, as well as chemical interactions between the metal and the ions in the electrolyte. To identify the relative impacts of various impedance components at different frequencies, an electrical equivalent circuit is developed for the electrode pairs under test, as shown in Fig. 1.

In Fig. 1, R_lead represents lead resistance, which arises from the thin film metal lines, bonding pads, etc., therefore they are in series with the electrolytic cell. R_lead is calculated to be 15~18 Ω for Al and Ti/Au electrodes. C_cell accounts for direct capacitive coupling between the two electrodes. The value of C_cell depends on the dielectric properties of the electrolyte and electrode geometries. The bulk of the electrolyte obeys Ohm’s law, so the bulk of the solution is modeled as a resistor R_solu in series with components at the interfaces of the electrodes and the electrolyte.
There are several current conducting mechanisms at the electrode-electrolyte interface. Hydrolyzed ions at the surface of metal electrodes cause a double layer capacitance, \( C_{dl} \). There are also electrode reactions at the interface, which is represented by a Faradic impedance in our equivalent circuit. If there is an electrode reaction, electric charges are transferred across the interface in parallel to the charging of the double layer, so the Faradic impedances are in parallel with \( C_{dl} \) (see paper by Ben et al. from our group in these proceedings). Faradic impedances are typically modeled as a battery (for the DC half-cell potential), an electron-transfer resistor and a Warburg impedance (for ion diffusion) in series.

The relative importance of circuit components determines frequency response of the electrode impedance. Because of small thickness of double layers, \( C_{dl} \) is generally much larger than \( C_{cell} \). Therefore, at low frequencies, voltage drops mostly happens at the electrode-electrolyte interface, and the cell impedance behaves somewhat like a capacitor. However, due to electrode reactions, there is resistive component, so the Bode plot deviates from a -20dB/decade (or -decade/decade) downward slope. As frequency increases, the admittance across the double layer becomes smaller and more current flows through the resistive bulk of the solution, so the impedance spectrum approaches a flat line, and that is the bulk resistance, \( R_{sola} \). At higher frequencies, the dielectric coupling of the electrodes dominates the cell impedance, and the impedance curve becomes a -20dB/decade line again. It can be deducted that only when \( R_{sola} \) dominates the current conducting path, can sufficient voltage be applied over the electrolyte and can DEP take place.

The time-averaged dielectrophoretic force, \( F_{DEP} \), acting on a homogeneous, isotropic dielectric sphere, is given by [12]

\[
F_{DEP} = 2\pi r^3 \varepsilon_m \text{Re}[K(\omega)] \sqrt{E_{rms}^2 - \frac{1}{(j\omega C_{cell})}}
\]

where \( r \) is the particle radius, \( \varepsilon_m \) complex permittivity of the suspending medium, \( E_{rms} \) the rms electric field, and \( K(\omega) \) is the Clausius-Mossoti factor, given by

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

where \( \varepsilon_p^* \) and \( \varepsilon_m^* \) are the complex permittivities of the particle and medium, respectively, and \( \varepsilon^* = \varepsilon - j(\sigma/\omega) \). According to eqs. (1) and (2), the DEP force depends not only on the strength of the applied electric field, but also on the field frequency. From an engineering point of view, the highest efficiency can be obtained if \( \varepsilon_m \text{Re}[K(\omega)] \) reaches its maximum at a frequency when \( R_{sola} \) dominates the cell impedance.

One point of note is that particles in the current path may modify the impedance of the solution. Even with latex particles, a Stern layer of adsorbed ions and a monolayer of ionic or polar surfactants can exist on their surfaces to endow the particles with a surface conductivity higher than the solution [13]. The resulting reduction in conductance could in turn change the potential drop across the electrolyte and consequently DEP force if the oscillation frequency is close to crossover frequencies.

**RESULTS AND DISCUSSIONS**

Figure 2 shows the impedance spectra of the electrolytic cells with Al and Ti/Au electrodes for suspensions with and without latex particles, respectively. It can be noticed from Fig. 2 the three regions discussed in the previous section: Below 1 kHz electrode-electrolyte interface properties dominate, between 1 kHz and 200 kHz, bulk electrolyte dominates and above 200 kHz electrode capacitance takes over. The approximate values of circuit elements in Fig. 1 can be extracted and they are given in Fig. 2. The capacitances can be found by fitting the impedances to a -20dB/decade line and extrapolating it to intercept the ordinate. The measured curves deviate at low and medium frequencies from a calculated curve using ideal circuit elements, because the Faradic currents and frequency-dependence of electrolyte conductivity have not been accounted for. Above 500 kHz, the cell behaves like a pure capacitor, i.e. \( C_{cell} \) bypasses all the applied electric field.

Fig. 2 Bode plot of impedance spectra with two types of electrodes and DI water with and without particles

It is worth noting the changes in impedance spectra after the latex particles are added to the solution for Al and Ti/Au electrodes. The impedances were noticeably reduced for measurements with Al electrodes, while there are slight increases of impedances with Ti/Au electrodes. The reduction in the cell impedances with Al electrodes can be explained by positive DEP. Due perhaps to the Stern layer or the surfactant layer, latex particles tend to exhibit a higher surface conductivity than DI water, so the cell
impedance decreases when the particles are attracted to the electrodes. The reduction becomes larger at higher frequencies partly because higher field is applied to the solution, and also because the particles experience steady positive DEP in this frequency range. In contrast, no conclusive changes in impedance have been observed with Au electrodes for all latex particle concentrations. This disparity can be qualitatively accounted for with different electrode processes at the Al and Au electrodes. Au, as a highly polarizable material, may develop an AC polarization opposite from the latex particles and inhibit DEP trapping of the particles [10].

If the detection of particles is realized through the change of solution conductivity, it is important that the cross-over frequencies be separated, i.e. there should be a frequency window where the solution conductivity controls the spectrum. Large electrode capacitance, \( C_{\text{cell}} \), can smear out that region and render detection inefficient. Figure 3 provides such an example. The positive DEP is exhibited, but less maximum difference in impedance can be obtained than from Fig. 2, because \( C_{\text{cell}} \) started to short out the current paths in the cell at higher frequencies.

CONCLUSIONS

The paper presents experimental results on micro EIS, and demonstrates the application of AC electrokinetic techniques for the manipulation and characterization of particles. Other than dielectrophoresis as a possible means of trapping the particle and enhancing the sensitivity of the EIS sensor, electrode polarization and subsequent particle repulsion are shown to have an opposite effect. These undesirable new electrokinetic phenomena are especially prevalent for micro-electrodes with small particle-electrode and electrode-electrode separation. The comparison between Al and Au electrodes underscores this important new dimension for the design of on-chip electrical detectors.

REFERENCES