Electrochimica Acta 280 (2018) 191–196

Contents lists available at ScienceDirect

Electrochimica Acta

journal homepage: www.elsevier.com/locate/electacta

Electrokinetic preconcentration and electrochemical detection of *Escherichia coli* at a microelectrode

Ariana Frkonja-Kuczin, Louis Ray, Zhiling Zhao, Michael C. Konopka, Aliaksei Boika*

Department of Chemistry, The University of Akron, 190 E. Buchtel Common, Akron, OH 44325, USA

ARTICLE INFO

Article history:
Received 30 January 2018
Received in revised form
16 May 2018
Accepted 17 May 2018
Available online 18 May 2018

Keywords:
Stochastic electrochemistry
Single entity detection
Dielectrophoresis
Electrothermal fluid flow
Escherichia coli

ABSTRACT

In this paper, we describe a novel electroanalytical approach that utilizes preconcentration of *Escherichia coli* (*E. coli*) bacteria by electrokinetic phenomena (dielectrophoresis and electrothermal fluid flow), which are generated in an electrolyte solution by a high frequency alternating current (ac) waveform. The bacteria are decorated with silver nanoparticles, so it becomes possible to detect individual bacterial cells one by one, i.e., perform electrochemical counting, due to silver oxidation on a working microelectrode (UME) biased at an appropriate dc potential. We demonstrate that the developed methodology allows for detection of *E. coli* concentrations two orders of magnitude smaller than what one could detect based on the transfer of *E. coli* by diffusion only (Sepunaru et al., Biomater. Sci., 3 (2015) 816–820).

1. Introduction

At the center of sensor technology lies a fundamental principle that the most sensitive analytical measurement is the one involving a single analyte entity, whether it is a molecule, an ion, or a nanoparticle (NP). Thus, one cannot do any better in terms of the sensitivity but detecting individual analyte species one by one, i.e. performing analyte counting [1]. In regards to this, methods of stochastic electrochemical detection (electrochemical collisions or electrode impacts), developed initially by Heyrovsky [2] and Scholz [3], and later by Bard and Compton groups [4–6], offer an unprecedented sensitivity that reaches a single entity level. These methods can be, in general, classified as direct and indirect. 'Direct' means that the species are transformed at the electrode directly (such as the oxidation of Ag NPs), while 'indirect' means that the collisions of the analyte species result in the observable changes in the electrochemical reaction that does not involve the analyte directly (such as in electrocatalytic amplification and blocking collisions).

The need for detection of individual analyte species requires the use of very small (~10 μm and below) indicator electrodes possessing relatively small background currents. This, however, leads to a fundamental complication, observable at ultra-low concentrations, that an analyte needs to travel sizable distances before arriving at the electrode surface. Therefore, if one relies on diffusion as the dominant mode of mass transfer, this translates into impractically long analyses times, or not so low detection limits (~pM, picomolar, 10^{-12} M). For a point-of-care detection, the issue of time becomes particularly important; generally, one would like to avoid the analysis time longer than 1–10 min due to the possible electrode fouling. A partial solution has been realized by employing electrophoretic migration, or directed motion of charged analytes under the action of an electric field, which has been demonstrated in the detection of polymer beads, metal NPs and virus particles in the sub-pM – fM (femtomolar, 10^{-15} M) range [7–10]. We have also developed a novel approach to estimation of the analyte concentration that is based on the time of first arrival (TFA), the period of time between the beginning of analysis and the moment of observation of the first collision event [11]. Based on this approach and employing electrophoretic migration, one could estimate the analyte concentration down to ~10 aM (attomolar, 10^{-18} M). The limit of detection and first passage statistics in the case of diffusional impacts of nanoparticles on microdisk and microwire electrodes have been also considered by Eloul et al. [12].

A number of analytical techniques are available for detection and quantification of bacteria, including optical density (OD) and microscopic measurements, cell culturing, electrochemical impedance techniques, and, most recently, methods of stochastic

* Corresponding author.
E-mail address: aboika@uakron.edu (A. Boika).

https://doi.org/10.1016/j.electacta.2018.05.120
0013-4686/

electrochemical detection [13–17]. Although each of these techniques possesses unique advantages, there are certain limitations precluding them from the wide application. One could argue that spectroscopic OD measurements, although simple and fast, are not sensitive enough, while microscopic counting is tedious and time-consuming. Cell culturing also takes a long time, sometimes up to 5 days. Similarly, electrochemical methods based on impedance measurements are not fast, typically taking at least one hour to complete to achieve low detection limits. Recent reports describing stochastic electrochemical detection of bacteria have some drawbacks: detection of diffusing *E. coli* leads to detection limits on the order of picomolar (pM, 10^{-12} M) [16]. Relying on electrophoretic migration leads to lower detection limits (~50 fM, femtomolar, 10^{-15} M), but at the expense that only dilute electrolyte solutions can be used [17]. It should be noted, however, that the composition of human's body fluids is complex, with concentrations of various ions on the order of $\geq$100 mM [18]. This makes it hard to utilize electrophoresis for analyte preconcentration under physiological conditions. Therefore, it is proposed here that we turn our attention to other electrokinetic phenomena, such as dielectrophoresis (DEP) and electrothermal fluid flow (ETF), due to some advantages they can bring to the field of bioelectroanalysis.

DEP is the phenomenon of a translational motion of dielectric particles under the action of a nonuniform electric field. Like electrophoresis, the movement is driven by the electric field. The difference is that the particles are not required to be electrically charged; rather, the DEP force, leading to the motion, acts on polarization charge (dipole) formed in the particles under the action of the electric field. The use of DEP for manipulation of various particles has been pioneered by Pohl in 1960s [19,20]. Since then it has been applied to sorting and separation, without any labeling, of biological cells, bacteria, viruses, DNA and RNA macromolecules, and proteins [21–26]. We have observed and described electrokinetic phenomena such as the DEP and ETF at microelectrodes polarized by a high frequency ac waveform ("hot microelectrodes") [27–29]. Unlike DEP, ETF sets in due to the presence of both the electric and temperature gradients in solution. Both phenomena can affect the mass transfer of analytes: DEP acts directly, as described above, while the ETF can drag analyte particles in solution, thus leading to their motion.

In this paper, we report on a methodology for bacterial (*E. coli*) detection that is highly sensitive and without limitations mentioned above with respect to the concentration of the supporting electrolyte. *E. coli* has been previously immobilized, patterned, and sorted using DEP [30–32]. Whether the DEP force pulls a particle towards or away from the electrode surface depends on the frequency of ac waveform applied as well as the permittivity and conductivity of the solution, and is determined by the real part of the Clausius-Mossotti factor (CMF). Rahmani et al. used a three-shell non-spherical model of the CMF to show that *E. coli* is pulled away from the electrode at a frequency of 100 MHz in pure water [33]. Recently we described the use of ac electrokinetic phenomena (DEP and ETF) for manipulation of individual Ag and Pt NPs in combination with their stochastic electrochemical detection [34]. Here we expand the proposed approach to include a new analyte – *E. coli* – that is decorated by Ag NPs similar to the report by Sepunaru et al. [16]. The role of Ag NPs is twofold: they serve as redox tags, as well as facilitate the manipulation of the bacteria by DEP. It should be noted that a number of groups combined DEP and ETF manipulation of analytes, including bacteria [35,36] and biomolecules [37,38], to improve their preconcentration and detection in a microfluidic channel. However, the detection of the analytes in those cases was not based on the methods of stochastic electrochemistry and was not done on a single UME. Finally, it is worth mentioning that, most recently, microfluidic impedance cytometry [39] and electrorotation [40] techniques have been applied to the analysis of bacterial analytes. Such powerful techniques, however, are less favorable for portable sensor applications, for which the stochastic electrochemistry approach could be a better candidate.

2. Experimental

2.1. Materials and instrumentation

All experiments were performed in an electrochemical cell consisting of a 0.3 mm platinum wire acting as a pseudoreference electrode (its potential was determined to be *ca.* −0.18 V *vs.* Ag|AgCl|KCl(sat.)), a counter electrode made of 0.5 mm tungsten rod, and an 11 μm diameter carbon microelectrode purchased from Bioanalytical Systems, Inc. The working electrode was polished using 1.0 and 0.3 μm MicroPolish Alumina on a soft pad (MicroCloth, PSA, 2.875 in. from Buehler). Solutions were prepared using nanopure water purified using a Milli-Q Integral 5 water purification system (Millipore, Bedford, MA) and ACS grade chemicals. The instrumental setup consisted of a commercially available potentiostat (model 760E, CH Instruments, Austin, TX), which interfaced with an MXG analog signal generator (Keysight Technologies model N5181B) through a low-pass filter. The design of the filter was the same as reported in previous publications from our group [34,41]. The ac signal generator had nominal power output levels from −144 dBm to +26 dBm (at 1 GHz) and the frequency range from 9 kHz to 3 GHz. The ac waveform was applied between the counter and working electrodes. The electrochemical cell and the filter were positioned inside a grounded Faraday cage (CH Instruments) to minimize the effect of external electromagnetic interferences on the experimental data.

2.2. Synthesis of Ag NPs

Citrate-capped Ag NPs were synthesized using the procedure described by Bastús et al. [42], and the average diameter and concentration were determined by analysis using a Nanosight NS500 instrument from Malvern, USA (Figs. S1–S2 in the Supporting Information). The first batch of Ag NPs were found to have an average diameter of 27.3 ± 0.1 nm and a concentration of 3.53×10^{11} particles per mL. These were used in the experiments of 2.5 and 25 fM (1.5×10^{6} and 1.5×10^{7} CFU/mL) *E. coli* (note that 1 CFU is not necessarily equal to one bacterial cell; however, we convert CFU/mL to molar concentrations assuming 1 CFU = 1 cell in order to calculate the concentration of Ag NPs needed to decorate the surface of bacteria). The second batch of Ag NPs used in the 80 and 125 fM (4.8×10^{7} and 7.5×10^{7} CFU/mL) experiments were found to have an average diameter of 27.2 ± 0.0 nm and a concentration of 1.45×10^{11} particles per mL. Ag NPs were washed by centrifuging at 14,000 × g for 25 min and then decanting the supernatant and replacing with water. After two washings, Ag NPs were concentrated to 3.53×10^{13} and 1.45×10^{13} particles per mL by centrifuging at 14,000 × g for 25 min and then removing liquid.

2.3. E. coli growth procedure and cell viability experiments

MG1655 wild-type *E. coli* obtained from the Konopka lab at The University of Akron were cultured in MOPS minimal media (0.4% glucose) and were incubated at 37 °C in cell culture tubes in a Barnstead Lab-Line Imperial III Incubator (Model 310) with shaking.

2.3.1. Growth procedure

50 μL of stationary phase cells were injected into 5 mL of sterile MOPS minimal media (0.4% glucose). Samples of the culture were taken at different time intervals over 13 h, diluted ten times with