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Zeta potential of bacteria cells: Effect of wash buffers

Wenfa Ng (Presenter) and Yen-Peng Ting*
Department of Chemical and Biomolecular Engineering, National University of Singapore
4 Engineering Drive 4, Singapore 117576, Singapore
*Corresponding author, Email: chetyp@nus.edu.sg

Zeta potential, defined as the electric charge at the shear plane, is widely used as a proxy for bacteria cell surface charge. Nonspecific adsorption of ions or polyelectrolytes onto the cell surface, however, alters the value and polarity of the measured zeta potential, leading to erroneous results. Multiple wash and centrifugation steps are commonly used in preparing cells for zeta potential analysis, where various wash buffers (such as 9 g/L NaCl, 0.001M KCl, and 0.1M $NaNO_3$) are routinely used for removing (by charge screening) ions and charged molecules that bind nonspecifically to the cell surface. Preliminary data showed that, for *Escherichia coli* DH5α (ATCC 53868) grown in LB Lennox (with 2 g/L glucose, or LBG), the zeta potential-pH profile was not significantly different over the pH range from 2 to 12 for deionized water, 9 g/L NaCl, and phosphate buffer saline (PBS) wash buffers. As LBG is a low salt medium without a phosphate buffer, it was likely that the extent of nonspecific adsorption of ions on the cell surface was not substantial and the different wash buffers would correspondingly not affect measured zeta potential much. For *E. coli* grown in a semi-defined medium (with a high capacity phosphate buffer system), the zeta potential-pH profile was significantly different over the pH range from 1 to 12 for deionized water, 9 g/L NaCl, 0.1M $NaNO_3$, 0.1M sodium acetate, and 0.1M sodium citrate wash buffers with the extent of difference positively correlated with wash buffer's ionic strength. Furthermore, the point of zero charge (pH_{zpc}) for *E. coli* grown in the semi-defined medium varies between 1.5 and 3, in an ionic strength-dependent manner, for the various wash buffers tested. Collectively, this preliminary study suggests that wash buffers' ionic strength strongly affect removal efficiency of nonspecifically absorbed ions on bacteria cell surfaces, where a threshold exists (0.15M) for charge screening to be effective. At the upper bound, 0.6M ionic strength might result in removal of cations that stabilize the cell envelope; thus, leading to possible cell surface damage and erroneous measurements.

Keywords: zeta potential; wash buffer; surface charge; nonspecific adsorption; *Escherichia coli;*

Introduction

The charge on the bacteria cell surface is of fundamental interest given its roles in mediating adhesion processes in biogeochemical cycles, wastewater treatment and bioremediation. Zeta potential is a popular proxy for gaining a quick understanding of the net charge on a cell surface at a specific pH. The choice of wash buffers for the multiple wash and centrifugation steps in sample preparation, however, plays a very important role in determining measurement accuracy. This in-progress report illustrates this point through two model systems: *Escherichia coli* grown in a low salt medium, and in one with a high capacity buffer.

Material

The composition of the two media used in this study is as follows. LB Lennox (with 2 g/L glucose) [g/L]: Tryptone, 10.0; Yeast extract, 5.0; NaCl, 5.0; D-Glucose, 2.0. Semi-defined medium [g/L]: K_2HPO_4, 12.54; KH_2PO_4, 2.31; NaCl, 5.0; Yeast extract, 12.0; NH_4Cl, 1.5; D-Glucose, 6.0; $MgSO_4$, 0.24. Zeta potential was measured with Malvern's Zetasizer Nano ZS in the microelectrophoresis mode.

Experiment

Escherichia coli DH5α (ATCC 53868) was grown in LB Lennox (with 2 g/L glucose) and a semi-defined medium for 15 hours at 37 °C and 230 rpm prior to sample preparation for zeta potential measurement. An aliquot of the cell broth was diluted 16 times (final OD_{600nm} = 0.30)

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with the respective wash buffers and centrifuged at 3300 x g for 10 minutes at 25 ºC. After centrifugation, the supernatant was carefully decanted off, and the cell pellet resuspended in the respective wash buffer. This process was repeated two more times, with deionized water as the final resuspension buffer for all samples. 0.1M HNO_3 and 0.1M NaOH were used to adjust the pH of the samples. Ionic strength of the wash buffers were estimated by the Debye-Huckel approximation.

Results and Discussion

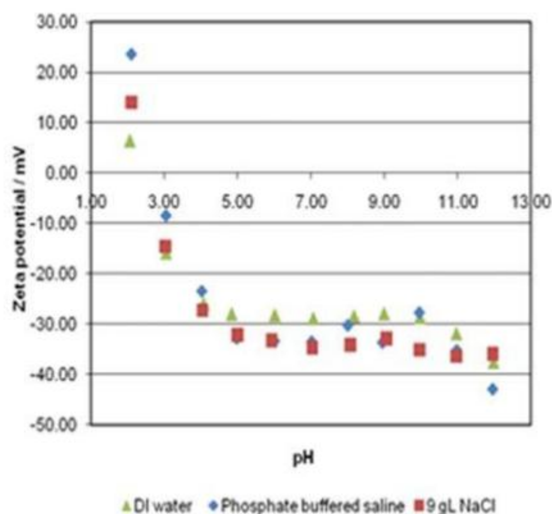

Fig 1: Effect of different wash buffers on zeta potential-pH profiles of *E. coli* grown in LB Lennox (with 2 g/L glucose). Larger version of figure is in supplementary information.

As can be seen in Figure 1, there was good overlap of the zeta potential–pH profiles for PBS and 9 g/L NaCl wash buffers. This suggested that charge screening played a major role in removing nonspecifically adsorbed ions from the cell surface since both PBS and 9 g/L NaCl have similar ionic strength of ~0.15M. It could also be observed that the differences between the three zeta potential–pH profiles were not very substantial. This could be due to small amount of nonspecifically adsorbed ions on the cell surface as LB Lennox (with 2 g/L glucose) is a low salt medium.

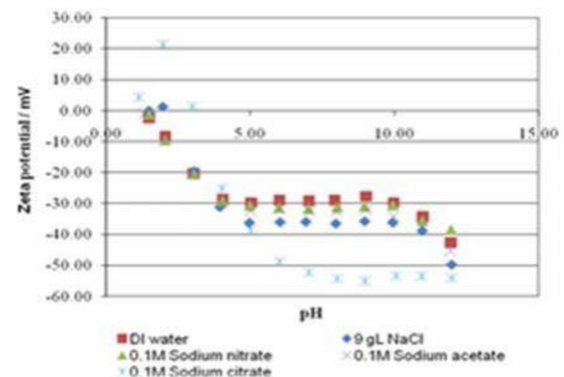

Fig 2: Effect of different wash buffers on zeta potential-pH profiles of *E. coli* grown in semi-defined medium. Larger version of figure is in supplementary information.

Figure 2 shows that the higher the wash buffer's ionic strength, the more negatively charged the cell surface was at pH 7, probably due to removal of cations from the cell surface. In addition, there was almost no difference in the zeta potential-pH profiles for deionized water, 0.1M sodium nitrate, and 0.1M sodium acetate, which suggested that a threshold ionic strength exists (in this case, at least 0.15M, e.g., 9 g/L NaCl) at which a wash buffer is effective in removing nonspecifically adsorbed ions. Finally, a wash buffer of 0.6M ionic strength (such as 0.1M sodium citrate) might drastically change the zeta potential-pH profile due to structural changes on the cell surface after washing.

Conclusions

Ionic strength of wash buffer plays an important role in determining measured zeta potential, primarily due to removal of nonspecifically adsorbed ions by charge screening. Specifically, an ionic strength of 0.15M seemed to be the minimum required for charge screening to be effective. On the other hand, an ionic strength of 0.6M would drastically alter the cell surface charge, and result in erroneous results.

Acknowledgment

The authors thank the National University of Singapore for financial support.

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