# Pickering Emulsion Polymerization of Polystyrene to Synthesize Bacteria Imprinted Polymer Beads for Pre-Concentration

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### Abstract:

*E. coli* is a fecal indicator that is useful to predict sanitary risk in water. Therefore, it is of interest to establish systems which are capable to monitor its concentration at realistic conditions. One possibility is to use molecularly imprinted polymers providing a selective surface binding attachment of *E. coli*. Bacteria imprinted polymer beads were synthesized via Pickering emulsion polymerization. In this process, *E. coli* served as a stabilizer to form a stable emulsion during radical initiated polymerization of styrene and DVB. Scanning electron microscopy revealed polymer beads before washing contain *E. coli* on their surfaces. After washing, imprints in the shape of *E. coli* are visible on the surface. Those beads are inherently useful for rebinding and thus selective filtration and pre-concentration of *E. coli* prior to sensing.

**Key words:** Bacteria Imprinted Polymers, Pickering Emulsion Polymerization, Polystyrene, *E. coli*, Fecal Indicator

# Introduction

Polluted water is a serious issue due to its health risk. Concentrations of pathogenic microorganisms are an important factor for water quality. *E. coli* indicates fecal contamination and is thus inherently useful to predict sanitary risk of a given water sample. This makes them an interesting target analyte in monitoring drinking water [1].

imprinted polymers Molecularly selective recognition on their surfaces, which is of potential use for the detection of bacteria [2], [3]. In Pickering emulsions, solid particles are used instead of surfactants to stabilize the emulsion [4]. As E. coli has the tendency to be located on the oil-water interface, this leads to a way for synthesizing MIP-beads with E. coli as a stabilizer of the emulsion [4]. The basic approach is sketched in Figure 1. Such bacteria-MIP beads are potentially useful for selective filtration and pre-concentration, which is a way to overcoming problems caused by low bacteria concentrations in real-life samples.

Herein, we report the synthesis of MIP-polystyrene beads using *E. coli* as a stabilizer of Pickering emulsion polymerization.



Fig. 1: E. coli imprinting by Pickering emulsion polymerization

## **Experimental**

*E. coli* W (ATCC 9637) was cultivated in a liquid culture medium for 22h at 37°C. To form a suspension, the bacteria were suspended in 4mL of distilled water.

MIP-beads were synthesized by mixing 0.5mL of styrene, 0.5mL DVB, 2wt% AIBN and 1.2mL of the *E. coli* suspension. Polymerization took

place at 37°C. *E. coli* was removed from the surface by solvent extraction using acetic acid and water. Bead surfaces were examined and characterized by scanning electron microscopy (Zeiss Supra 55 VP).

### Results

Indeed, *E. coli* turned out a suitable stabilizer to form a stable Pickering emulsion based on styrene and DVB as the oil phase. As Figure 2 shows, scanning electron microscopy revealed that indeed large amount of bacteria are attached to the surface of the beads after polymerization.

The following solvent extraction step required optimization. However, it turned out feasible to remove *E. coli* from the surfaces of the beads leaving behind the corresponding cavities on the surface, as shown in Figure 3B.

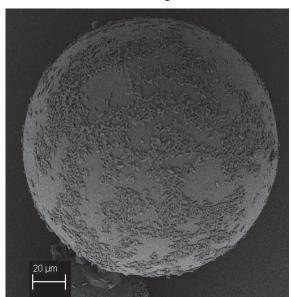


Fig. 2: SEM image of a bead with E. coli attached on its surface

### Discussion

MIP-beads are potentially able to selectively rebind *E. coli* on its surface due to the large number of imprints, which fits to the size and shape of the bacteria. This property could be exploited to perform filtration and preconcentration steps. If this process is combined with a detection system for *E. coli*, it could improve the monitoring of this fecal indicator.

# **Conclusion and Outlook**

Molecular imprinting in Pickering emulsions opens up a way for selective pre-concentration in sensing, which inherently tackles one of the main problems of sensing bacteria in real-life samples, namely low concentration of the target analyte.

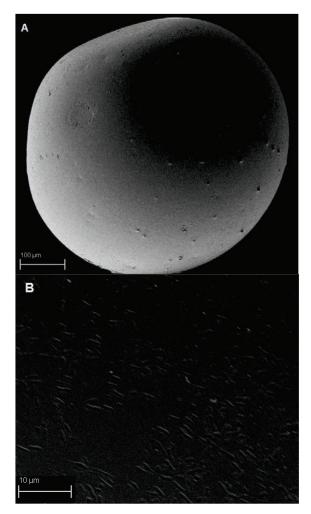


Fig. 3: SEM image of a bead after solvent extraction (A), SEM image of the imprints on the surface of the bead (B)

### References

- [1] A. De Brauwere, N. K. Ouattara, P. Servais, Modeling Fecal Indicator Bacteria Concentrations in Natural Surface Waters: A Review, *Crit. Rev. Environ. Sci. Technol.* 44, 2380-2453 (2014); doi: 10.1080/10643389.2013.829978
- [2] R. Samardzic, H. F. Sussitz, N. Jongkon, P. A. Lieberzeit, Quartz Crystal Microbalance In-Line Sensing of Escherichia Coli in a Bioreactor Using Molecularly Imprinted Polymers, Sens. Lett. 12 (2014) 1152-1155; doi:10.1166/sl.2014.3201
- [3] X. Shen, J. Svensson Bonde, T. Kamra, L. Bülow, J. C. Leo, D. Linke, L. Ye, Bacterial Imprinting at Pickering Emulsion Interfaces, Angew. Chemie Int. Ed., 53, 10687-10690 (2014); doi: 10.1002/anie.201406049
- [4] Y. Chevalier, M. A. Bolzinger, Emulsions stabilized with solid nanoparticles: Pickering emulsions, *Colloids Surfaces A Physicochem. Eng. Asp.*, 439, 23-34 (2013); doi: 10.1016/j.colsurfa.2013.02.054