

## Flocculation of a bacterial suspension by a cationic polyelectrolyte

Xavier Châtelier, Jean-Yvès Bottero and Jean Lepetit

CEREGE, Aix-en-Provence and University of Marseille, France

### Introduction

a) Context: The physico-chemical properties of bacterial surfaces are still quite poorly understood, although they play an important role in many processes (infections, biofilms formation, water purification etc). These properties are controlled by specific interactions, but also by short-range or long-range more general interactions. The long-range interactions may be of electrostatic nature, but can also be mediated by macromolecules. The presence of exocellular polymers is for instance known to be determinant for the formation of biofilms or for the flocculation of bacterial suspensions. In some other situations the polymers produced by the bacterial cells prevent them from interacting with other particles.

b) General methodology: Simplified systems may be useful to understand better the many mechanisms which may be involved in the various situations that can be encountered. Focusing on the problem of bacterial flocculation, we examined a model system consisting in a suspension of a pure bacterial strain (*Escherichia coli* B4) and of a synthetic polymer (quaternized polyvinylpyridine: PVPQ). The aim of our work is to understand how the polymer adsorbs on the bacterial surfaces, in which conditions the bacterial surfaces stick to each other, due to the presence of the polymer, and what are the properties of the bacterial aggregates in terms of size and structure. Noting that these questions have been investigated by many authors in the last two decades for abiotic colloidal suspensions (clays, silica, latex etc.), we have based ourselves on the assumption that their methods and results may be adapted to "biocolloids" like bacteria.

c) The model system:

Polymers: the polymers are positively charged. Their degree of polymerization is on the order of 5000, and the degree of quaternization is on the order of 80%. The Odijk-Skolnick-Fixman theory predicts that the persistence length of such chains is about 8 nm, leading to a gaussian radius of gyration in solution on the order of 200 nm, and to an overlap concentration of a few tenths of mg/l. The surface of a cell can be covered by at most about 100 non overlapping chains.

Bacteria: the cells are extracted from the growth medium while in exponential phase and washed 6 times in ultrapure water with added NaCl at an ionic strength of  $4 \cdot 10^{-3} \text{ M}$ . Their surface carries a negative charge. The zeta potential was measured and we obtained  $-47 \text{ mV}$ , suggesting an effective surface charge density at the shear plane of  $8 \cdot 10^{-3} \text{ C} \cdot \text{m}^{-2}$ . The surface area of one bacterium is about  $6 \cdot 10^{-12} \text{ m}^2$ .

### Adsorption of the polymer on the surface

a) Adsorption isotherm: PVPQ is a polymer which has the characteristics of absorbing strongly the light at 225 nm. We used this property to determine its adsorption isotherm on the bacterial surfaces. The polymer was added to the bacterial suspension in increasing quantities. After gentle stirring for 21 hours, the samples are centrifuged and the supernatant is analyzed by spectrophotometry. The spectrum of the optical density in the suspension allows an estimation of the remaining concentration of polymer, and thus of the quantity of non adsorbed polymer. The quantity of adsorbed polymer can then be estimated.

Results: the adsorption isotherm shows that the bacterial surfaces can adsorb large quantities of polymer, on the order of 40 mg/g, namely about 5000 chains on each cell. These quantities are comparable with usual values for clays, latex etc

b) Electrophoretic mobilities: the polymer was added to the bacterial suspensions as mentioned above. After the stirring period, sedimentation of the aggregates was allowed for one to two hours. The supernatant was diluted and analyzed with a zetasizer (Malvern 3000). The electrophoretic mobilities were interpreted in terms of the zeta potential, i.e. the electrostatic potential at the shear plane, using the Schmoluchowski approximation of the Henry formula. The measurements show that the adsorption of the polymer results in a charge inversion of the cells.

c) Interpretation: the isoelectric point corresponds to adsorbed quantities on the order of 30 mg/g, suggesting