EUROPEAN NETWORK FOR GASTROINTESTINAL HEALTH RESEARCH

EUROPEAN SCIENCE FOUNDATION

European Network for Gastrointestinal Health Research

Exchange Visit Report

Philippe Emmanuel da Cruz Ramos

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Universidade do Minho
Escola de Engenharia

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1 - Purpose of the visit

My name is Philippe Ramos, I am a PhD student in the Centre of Biological Engineering, University of Minho, Braga, Portugal. My supervisors are Professor António Vicente and Professor José Teixeira. My PhD is related with the microencapsulation of probiotics, capable of a high production of extracellular folate. Folate is one of the few vitamins that is not produced by our organism, which brings a huge interest of is presence on Human Diet. Although some factors exist that limit probiotics action in the human body: natural defenses reduction in case of people with some allergic disease or inflammatory bowel disease (IBD), very weak resistance in digestive juices contact, and low residence time in the intestine.

The main objective of my PhD is to immobilize a probiotic consortium in a microcarrier capable to pass throughout the whole gastrointestinal system without releasing probiotics, while promoting the exchange of nutrients and products (probiotic activation) in the intestine, by changing the porosity of a membrane sensitive to medium pH. After the development of microcarriers they will be introduced in a model food and their behaviour studied in an artificial gastrointestinal system. All the variables that influence this kind of systems, such as: number of layers, different pH's, temperature, different compounds, and also the size of the structure make of this evaluation a very hard task. Because of that, the collaboration with the Department of Food, Chemistry and Pharmacy at the University of Reading, where Professor Dimitrios Charalampopoulos, Professor Vitaliy Khutoryanskiy and the Researcher Michael Thomas Cook have a high expertise in this area, was an idea that came naturally regarding our difficulties and some of theirs publications about the microencapsulation of probiotics.

The purpose of this visit was to increase and diversify the characterization of the microcarrier and be able to learn more techniques and technologies that will be very important in the continuation and conclusion of my PhD plan. It was also a very important for the exchange of ideas regarding the next steps of my work.

2 - Description of the work carried out during the visit

The point of situation of my work when I arrived to Reading was:

- I had created the structure and defined the materials (alginate CR 8223 based microemusion with three different layers, poly-L-lysine (first layer), alginate LFR5/60 (second layer) and chitosan (third layer)) and the function of which one in the system as the optimized the concentrations of them.

The work carried out in this month at Reading was:

- Explain my microcapsule structure to my supervisors in the host institution, and create a work plan more adapted to the recent structure developed;
- Turbidity tests between the materials used;
- Selection of the chemicals to label the materials used, concerning that they needed to be different and have different emission wavelengths;
- Labelling Alginate and Poly-L-lysine with Fluorescein;

3 - Description of the main results obtained

Turbidity tests were performed to understand how the three different compounds interact each other. The different materials/different layers that were used were alginate CR 8223 (Higher amount of the mannuronic acid, which creates a more permeable layer), poly-L-lysine (capable of a high degree of cell adhesion), alginate LFR5/60 (Higher amount of glucuronic acid which creates a stronger net more able to resist to the acidic environment of the stomach) and chitosan (to improve the resistance of the particle to the acidic environment of the stomach).

To prove the adhesion of each layer, turbidity tests were performed between the different layers. The first experiment was performed with alginate CR 8223 and poly-L-lysine. The results showed a better interaction between the biopolymers at pH 4 and 6 (Figure 1). These results can be justified considering the *p*Ka of alginate (3.3) and poly-L-lysine (7.5 to 11) that correspond to a loss, or a decrease, of charge of these materials in these pH ranges. The interactions between these materials in this system are mainly created through the affinity of the charges between the molecules (alginate – negative charge; poly-L-lysine – positive charge). With these results it is possible to conclude that the interactions between these two materials are stronger in the range of pH 4 to 6. This is because the higher the difference between opposite charges (of a positively charged material and a negatively charged material), the stronger the interactions created and in consequence larger polysaccharide agglomerates are built that present higher turbidity in the spectrophotometry measurements.

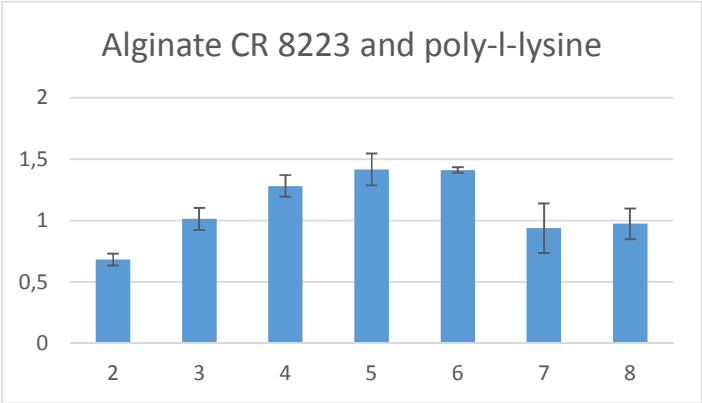

Figure 1 – Turbidity measurement between Alginate CR 8223 and Poly-L-lysine

The same experiment was performed with poly-L-lysine and alginate LFR5/60, the first and second layer of the microcapsule, respectively. These results show that results obtained for alginate CR 8223 and LFR5/60 have a similar behavior when in contact with poly-L-lysine (Figures 1 and 2).

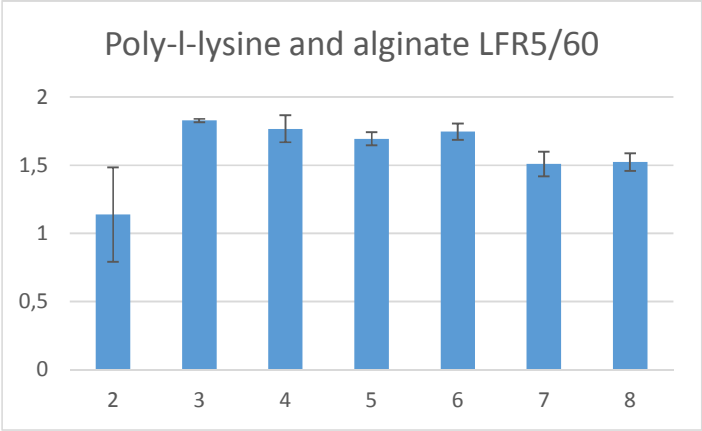

Figure 2 – Turbidity measurement between Poly-l-lysine and Alginate LFR5/60.

The measurements performed with Alginate LFR5/60 and Chitosan showed that the *p*Ka, as mentioned before, influenced the results. The *p*Ka values of alginate and chitosan are 3.3 and 7.6 to 11, respectively, which creates strong interactions between the biopolymers at pH values between 4 and 5. However, results showed stronger interactions when performed at pH 3; the reasons for this behaviour are still unclear and will have to be scrutinised in the following work.

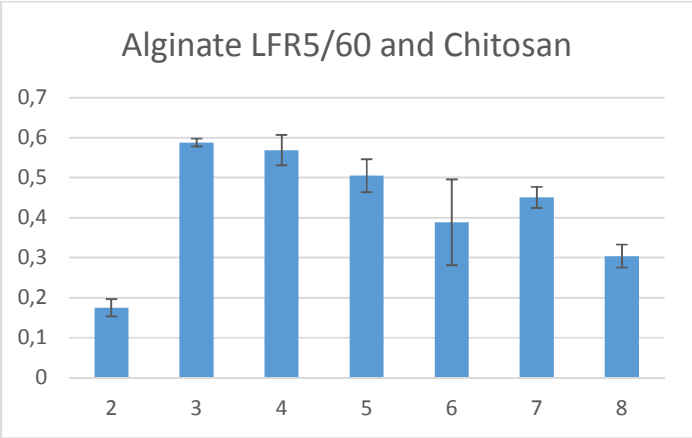

Figure 3 – Turbidity measurement between Poly-L-lysine and Alginate LFR5/60.

Results were important to demonstrate the existence of interactions between the biopolymers used, which is a good indicator of the formation of a layer-by-layer structure in the microcapsule. The next steps of this work will be the labeling of each layer and following the construction of layers at different pH values by confocal microscopy. The labeling of the materials was already started in this period but not finished considering the timings needed for labeling, freeze-drying and the frequency of the course in confocal microscopy. These tests will therefore proceed after this period.

4 - Future collaboration with host institution

Regarding future collaborations between the two institutions, Centre of Biological Engineering and Department of Food, Chemistry and Pharmacy, this visit was a profitable experience to both parts. Besides all the inputs for my PhD work it was also an opportunity to discuss and establish other works and ideas. A visit of one of my supervisors, Prof. Vicente, was very important to get closer relations and discuss the possibility of the two groups working together. It was also discussed the possibility of a student of the host university going to Portugal to do an exchange visit. The discussion of new ideas, different technologies and techniques used by the two groups were very important, not only in the development of my work but also for other works that were discussed in this meeting, including the participation in a project proposal to be submitted to the H2020 program of the EU. This exchange visit potentiates future collaborations that will be very important to the involved groups.

5 - Projected publications / articles resulting or to result from the grant

This period was important to plan the structure of a review article in this area and include the results presented above in an original article. Both articles will be written during the next year.