UNIVERSITY OF SALERNO

DEPARTMENT OF CHEMISTRY AND BIOLOGY "A.ZAMBELLI"

Ph.D. Course in Chemistry
XXXII Cycle

ABSTRACT

Dynamical control of engineered stimuli-responsive materials: synthesis and optimization of active micro-vesicles

Supervisor
Prof. Antonio Proto

Ph.D. Student
Ylenia Miele

Co-Supervisors
Prof. Federico Rossi
Prof. Lorella Izzo

External Supervisor
Dr. Annette Taylor

Ph.D Course Coordinator
Prof. Riccardo Zanasi

Academic year: 2018-2019

ABSTRACT

The aim of this PhD project is the combination of a pH clock reaction (the enzymatic urea-urease reaction) with pH-responsive materials to generate a chemo-mechanical coupling. A clock reaction is characterized by a latent period during which no significant change takes place in the mixture, and then a change occurs suddenly.[1] The urea-urease reaction[2,3] is a biocompatible pH sensitive reaction frequent in numerous cellular systems and used by bacteria *H.pylori* to raise the local pH according to the overall stoichiometry

$$CO(NH_2)_2 + H_2O \xrightarrow{\text{urease}} 2NH_3 + CO_2 \quad (1)$$

This reaction shows a bell shaped rate-pH curve with a maximum at pH 7 (Figure 1 a). Since one of the products (ammonia) is basic, if the initial pH is adjusted to a lower pH than the optimal one, then the reaction accelerates as it proceeds leading to a clock behaviour (Figure 1 b).[4]

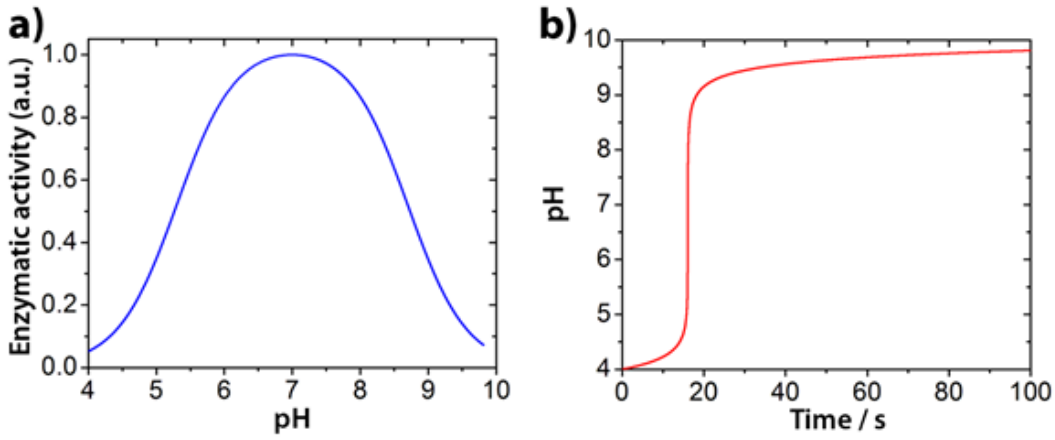

Figura 1: The characteristics of the urea-urease reaction. a) Dependence of the urea-urease reaction rate upon the pH in unbuffered solutions. b) autocatalytic profile of the pH during the urea-urease reaction.

pH-responsive materials alter their chemical and physical properties upon change in pH and for this reason are studied for a diverse range of applications: on-demand drug delivery, smart coatings, biosensing and analytic chemistry.[5,6] For example, the urea-urease system was coupled with a Michael addition of a trithiol to a diacrylate to trigger fronts of polymerization, the features of this system are attractive for many materials chemistry applications in which an initial slow reaction is required before a rapid curing.[7]

In this project, the urea-urease enzymatic reaction was coupled with pH-sensitive giant vesicles (giant vesicles are micron-sized compartments that mimic several features of cell membranes). First, the reaction conditions were established in pH-stable 1-Palmitoyl-2-oleoylphosphatidylcholine (POPC) vesicles. The length of the induction period and the final pH were estimated at different chemical concentrations and different number of vesicles. Some experiments with pure POPC vesicles were performed under the supervision of Dr.

Annette Taylor (University of Sheffield) by means of confocal microscopy. Once studied the reference system, two kinds of hybrid pH-sensitive vesicles were produced and characterized: lipid/oleate vesicles (the pH-sensitive group is the COOH, negatively charged at high pH) and lipid/polymeric vesicles (positively charged at low pH for the presence of amine groups -$N(CH_3)_2$).

In proper conditions, lipid/oleate vesicles coupled with the enzymatic reaction can give rise to self-division phenomena: one vesicle is able to divide into two daughter vesicles after a pH change (due to the ammonia production) and osmotic stress. The self division triggered by an internal chemical stimulus can be of interest in the Origin of Life research field where fatty acid vesicles are harnessed as protocellular models.[8]

The amphiphilic copolymers chosen for the last part of this project are linear three components A(BC) copolymers where A is a hydrophilic block of methoxy-poly(ethylene glycol) (mPEG), BC is a copolymer containing the hydrophobic methyl methacrylate (MMA) and the pH sensitive 2-(Dimethylamino)ethyl methacrylate (DMAEMA).

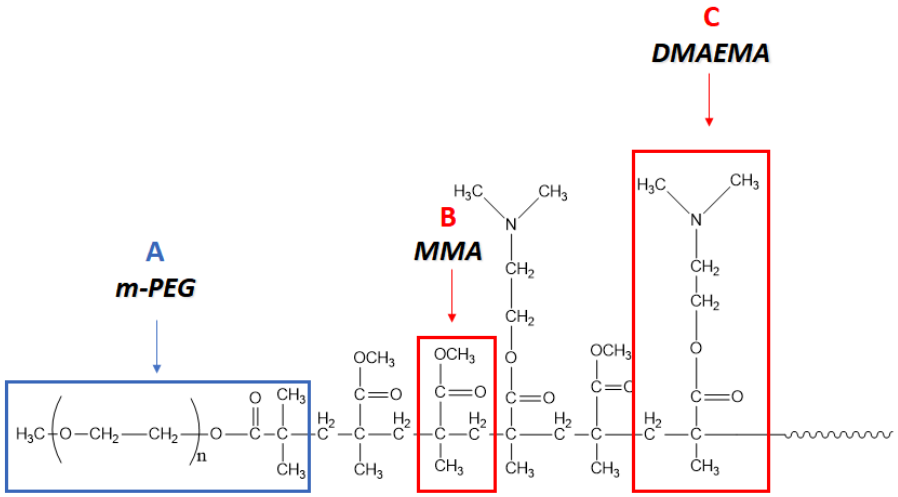

Figura 2: Structure of the copolymer mPEG-*b*-(PMMA-*co*-PDMAEMA).

In previous studies, these copolymers showed a strong swelling/deswelling pH-dependent behaviour when aggregated in pure polymeric nano-vesicles.[9] These results encouraged us to prepare and characterize polymeric giant hybrid vesicles made of POPC and mPEG-*b*-(PMMA-*co*-PDMAEMA). The DMAEMA based copolymers were synthesized through a living radical polymerization process (ARGET ATRP) and characterized in terms of molecular weight, composition and glass transition temperature: the pH-sensitivity of the copolymer selected for the vesicles preparation was verified through contact angle measurements. Mixed lipid/polymeric vesicles were prepared with two protocols: electroformation[10] and droplet transfer method.[11] Part of the project was conducted at IMRCP (University of Toulose Paul Sabatier). The droplet transfer turned out to be the best method for the formation of giant hybrid vesicles: the use of fluorescent markers allowed us to identify the presence of polymeric and lipid regions within the membrane.

Bibliography

[1] Shakhashiri, B.Z.: Chemical Demonstrations: A Handbook for Teachers of Chemistry. Volume 3. Univ of Wisconsin Press (1989)

[2] Krajewska, B., Ciurli, S.: Jack bean (Canavalia ensiformis) urease. Probing acid–base groups of the active site by pH variation. Plant Physiology and Biochemistry **43**(7) (2005) 651–658

[3] Bánsági Jr, T., Taylor, A.F.: Exploitation of Feedback in Enzyme-catalysed Reactions. Israel Journal of Chemistry **58**(6-7) (2018) 706–713

[4] Muzika, F., Bansagi, T., Schreiber, I., Schreiberova, L., Taylor, A.F.: A bistable switch in pH in urease-loaded alginate beads. Chemical Communications **50**(76) (2014) 11107–11109

[5] Wei, M., Gao, Y., Li, X., Serpe, M.J.: Stimuli-responsive polymers and their applications. Polymer Chemistry **8**(1) (2017) 127–143

[6] Lorenzo, R.A., Carro, A.M., Concheiro, A., Alvarez-Lorenzo, C.: Stimuli-responsive materials in analytical separation. Analytical and bioanalytical chemistry **407**(17) (2015) 4927–4948

[7] Jee, E., Bánsági, T., Taylor, A.F., Pojman, J.A.: Temporal control of gelation and polymerization fronts driven by an autocatalytic enzyme reaction. Angewandte Chemie International Edition **55**(6) (2016) 2127–2131

[8] Zhu, T.F., Szostak, J.W.: Coupled growth and division of model protocell membranes. Journal of the American Chemical Society **131**(15) (2009) 5705–5713

[9] Villani, S., Adami, R., Reverchon, E., Ferretti, A.M., Ponti, A., Lepretti, M., Caputo, I., Izzo, L.: pH-sensitive polymersomes: controlling swelling via copolymer structure and chemical composition. Journal of drug targeting **25**(9-10) (2017) 899–909

[10] Angelova, M.I., Dimitrov, D.S.: Liposome electroformation. Faraday discussions of the Chemical Society **81** (1986) 303–311

[11] Walde, P., Cosentino, K., Engel, H., Stano, P.: Giant vesicles: preparations and applications. ChemBioChem **11**(7) (2010) 848–865