ChemComm

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Journal:	*ChemComm*
Manuscript ID:	CC-COM-03-2014-002321.R1
Article Type:	Communication
Date Submitted by the Author:	16-Jun-2014
Complete List of Authors:	Pereira de Souza, Tereza; Harvard University, Earth and Planetary Sciences Perez-Mercader, Juan; Harvard University, Earth and Planetary Sciences

Journal Name

RSCPublishing

COMMUNICATION

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Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

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T. Pereira de Souza[a] and J. Perez-Mercader[a]

We present a methodology to entrap and run the Belousov-Zhabotinsky (BZ) reaction inside a polymer vesicle. We report on experiments with polymer vesicles where we observed (1) vesicle membrane deformations, (2) oscillations and (3) pulsating (expansion/contraction) episodes. The above provide us with evidence for chemo-mechanical coupling between these polymersomes and their contents.

The study of microscopic flexible containers for complex chemical reactions is central for understanding issues in the origin of life as well as in biotechnology[1-3]. In fact, natural questions that arise in the study of the dynamics of the interaction between chemical reactions taking place within flexible container vesicles (liposomes or polymersomes) and their walls (membranes)[4, 5], are (1) if and how the internal reaction couples to the container, and (2) how the effects of the internal reaction propagate into the collective dynamics of containers. Answers to the above questions are basic for the potential implementation of self-replication in vesicles, for the study of collective effects in the evolution of a population of vesicles as a consequence of their own internal chemical dynamics, and for biomimetic purposes when in search of simple pathways[6] for the synthesis of amphiphilic molecules without involving the fine-tuned multienzyme pathways that are required for the synthesis of liposome components[4] both in vitro and in vivo.

For example, one may ask how some cooperative behavior occurring in the chemistry inside a vesicle affects the behavior of the isolated vesicle itself as an individual in a population of vesicles, or if they induce any feedback or adaptive processes in the population. More specifically, when one considers an oscillatory chemical reaction contained within a vesicle we may also ask if the chemical oscillations couple mechanically (or otherwise) to the vesicle and, if so, what properties does this confer to the vesicle collective. In the following we present the results of experiments done to entrap by "self-assemblage" an oscillatory chemical reaction inside polymersomes with block copolymer membranes.

A prototype of an oscillatory chemical reaction is the well-known Belousov-Zhabotinsky (BZ) reaction[7, 8], while a typical class of substances used for vesicle formation are phospholipids[1, 3]. But, the BZ reaction takes place in a very low-pH medium (< 3), which is incompatible with the physico-chemical nature of most lipids[9, 10]. This incompatibility poses a difficulty in the study of potential container-contents relationship in pH oscillators and their stability. Motivated by these problems, we have asked what kind of materials could be used to entrap and contain a BZ reaction running in one of its oscillatory modes without using microfluidic vesicle fabrication techniques in order to force the entrapping. That is, to achieve the entrapment of BZ inside a vesicle in a somewhat primitive and autonomous "self-organized" manner. Because of the incompatibilities between the oscillatory reaction, the vesicles and their eventual self-organization, we have shifted our attention from lipids to block copolymers. They are known to be more robust than lipids to lower-pH[11], higher proton collision rates and other "extreme conditions"[9, 12, 13†]. We have focused on block copolymers known to be capable of forming vesicles with diameters in the range of a few microns to several tens of microns, that is, in the giant-vesicle size range[12, 14]. These polymers and their polymersomes have been extensively studied in the literature. The particular case of polybutadiene-b-polyethylene oxide (PB-PEO) is well characterized and its phase diagram is known to a sufficient level of detail so as to allow us to roughly estimate what morphologies would be formed in an aqueous medium as a function of the degree of polymerization of polybutadiene (N_{PB}), weight fraction of polyethylene oxide (W_{PEO})[15-17] and concentration of copolymer. This enables one to choose the blocks in the copolymer so that the formation of pH-resistant vesicles based on PB-PEO[11] is guaranteed.

Armed with the above, we have carried out a series of experiments trying to explore which combination of vesicle material (including polymer conformation, vesicle forming procedure, and the BZ recipe used in the experiments) and avoiding the use of microfluidic extrusion techniques could generate giant polymersomes in pure

water. Once the vesicles were formed, we proceeded to their observation and characterization.

As described in the supplementary information (ESI) we first performed our observations in a microscope slide on which we placed previously formed vesicles in 14 MΩ water and observed the evolution of their membrane, including swelling and deswelling due to thermal fluctuations, and on to their eventual evaporation. These experiments indicated that in pure water our polymersomes swelled (10% at 0.1 um/s) and shrank at a very slow rate [approx. 0.077um/s] by a small percentage [11.8%] of their size in a period of about 2 hours. After this induction period, the polymersomes eventually went through a sequence that included (a) shrinkage, (b) break up and (c) drying. This constrained our experiments to a maximum of 2 hours. Images of vesicles in a typical run under these conditions are shown in the ESI (Figure S1 and PolW1.avi) and demonstrate that our polymersomes do not oscillate in water; and that their membranes do not present any kind of pulsation or oscillation. These were our control experiments.

Our next round of experiments was designed to ensure that we could form polymersomes capable of entrapping the BZ reaction medium and that, additionally; we could observe their oscillations due to the BZ reaction running in their interior. We also looked for signs of any potential chemo-mechanical coupling between the reaction and the polymers in the vesicle membrane. For these experiments we took into account the well-known fact that chemical oscillations in the unstirred version of the BZ reaction have an induction period. This period depends on the particular concentrations of chemicals used in the reaction recipe[7, 18]. Since we were concerned about vesicle break up, and as we do not use any stirring inside the vesicle, we took a two-step approach to deal with potential vesicle disruptions and used two different vesicle formation protocols.

First we used the rapid evaporation method[19] (REM) for vesicle formation. We tuned our protocol so as to generate vesicles as quickly as possible while also taking into consideration the requisites imposed by the unstirred formula used for our MA-BZ (Malonic Acid version of the BZ, cf. ESI) reaction (Figure S2).

The REM allows the formation of PB-PEO vesicles in the MA-BZ reaction. However the same MA-BZ reaction mixture was both inside and outside the vesicle. This precluded the use of sucrose[††] in the external solution. To avoid this difficulty we changed the protocol for vesicle formation from REM to the inverted emulsion method[20, 21] (IEM).

However, when we started with the IEM we were not able to form vesicles. This was because of perturbations to the vesicle from the CO_2 bubbles generated in the MA-BZ reaction. In order to overcome this difficulty, we entrapped instead the CHD-BZ (Cyclohexanedione version of BZ, cf. ESI) reaction inside the PB-PEO vesicles.

Vesicles were formed inside a 100 mM sucrose solution. This ensured that we would actually be able to use microscopy to observe exclusively the potential oscillations due to the BZ reaction running in the interior of our polymersomes.

In the following we discuss of results of the above experiments. For the case of REM with MA-BZ reaction, we observed perfectly spherical vesicles until around 2 minutes after formation, which coincided with the end of the induction period for our MA-BZ reaction recipe (PolBZ1.avi and PolBZ2.avi). With the subsequent evolution of the MA-BZ reaction we observed that the membrane dynamics of the polymersome was completely different from the one in the control experiment. We can report the presence of sequences of inhomogeneous contractions and expansions of the membrane that occur in two distinct phases. The first or initial phase, takes around 25 minutes, and during it we observed a transient deformation in the shape of the polymersomes (Figure 1, B and C and Figure S3, B to D). Giant polymersomes with diameter of 15 µm, present strong changes in their geometry (Figure1), while 25 µm (Figure S3) to 60 µm (data not shown) giant polymersomes present less pronounced deformations. After this, a second phase ensued, starting around 25 minutes after the initiation of the experiment, and characterized by deformations of all vesicles in a manner gentler than during the first phase. During this phase the shape of the polymersomes did not change considerably. The second phase lasts around 1 hour. These results suggest the existence of a relationship between vesicle size and the intensity of any chemo-mechanical effect as detected by phase contrast microscopy. Smaller giant vesicles, in spite of their higher curvature, offer a more susceptible surface for deformations. These deformations were observed in the form of membrane invagination, thus suggesting to us that the reaction is synchronized inside and outside the polymersome. Observing Figures1 and Figure S3, which show the time evolution of (25 and 15 µm diameter) giant polymersomes in the MA-BZ reaction, and comparing the images labelled as A and F, corresponding to the observations at t=0 and t=90 minutes, we note that there is no evidence of membrane disruptions during the evolution of the vesicles. This suggests that the coupling between the polymer material and the MA-BZ reaction does not interfere destructively with the material forces intrinsic to the polymersome itself.

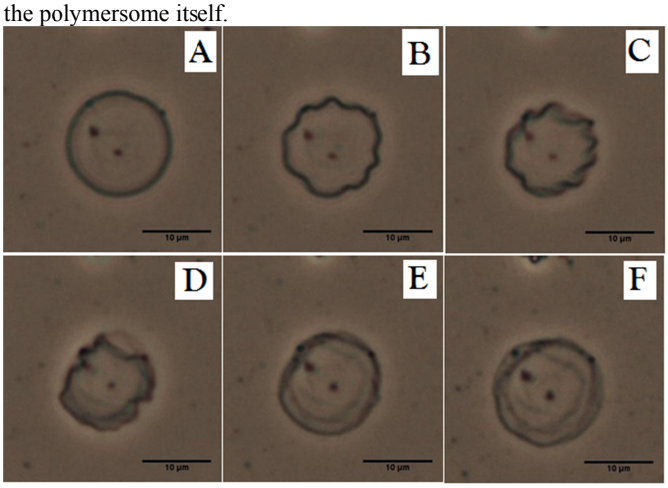

Figure 1: Polybutadiene-b-polyethylene oxide (PB_{46}-b-PEO_{31}) polymersome containing MA-BZ reaction (H_2SO_4 600 mM, malonic acid 70 mM, $NaBrO_3$ 100 mM, $Ru(bpy)_3$ 0.24 Mm) prepared using rapid evaporation method. BZ reaction is present inside as well as outside the polymersomes. The micrographs shown in panels A to F demonstrate coupling between the MA-BZ reaction and the polymersome membrane. Panels above represent the evolution of a particular vesicle. The time in seconds after the vesicle formation and the respective vesicle diameter sizes are: A (124 s; 15 µm); B (340 s, 14.4 µm); C (568 s, 12.8 µm); D (980 s, 13.1 µm); E (1524 s, 14.8 µm); and F (5120 s, 15.6 µm). Phase contrast. Scale bar 10 µm.

So far our results therefore indicate the presence of the full BZ reaction inside PB_{46}-PEO_{31} polymersomes with their BZ running in one of its oscillatory modes. However, in the previous experiments we also had BZ outside the vesicles, and the addition of sucrose for enhancing the contrast for microscopy complicates the procedure as BZ is perturbed by the presence of sugars. Encouraging as they are, these results cannot demonstrate that what we observe is exclusively a direct consequence of the BZ reaction entrapped within the polymersomes. But the above coupled with the CO_2 bubbles in the BZ reaction make it very difficult to detect the presence of any actual chemo-mechanical coupling.

We wanted to check for the presence of BZ in an oscillatory mode exclusively contained inside of the polymersome. The experiments

done using IEM with the CHD-BZ reaction resolve the previous difficulties: they allow us to have only sucrose in the external polymersome solution (cf. ESI) while only the CHD-BZ reaction solution inside the polymersomes[20, 21]. We found in all our preparations the formation of polymersomes, albeit with a heterogeneous size distribution: (1) small giant polymersomes observed as single vesicles or as pairs of similarly sized vesicles; (2) large giant polymersomes (approx. 30 μm in diameter) were preferentially present as clusters of 3 or more vesicles (PolBZ3.avi). These clusters were composed by a number of different size polymersomes with typical diameters ranging from a few microns to tenths of micrometers. Experimentally, we observed two distinct situations. First (PolBZ3.avi), each polymersome in the cluster undergoes two or three pulsation episodes followed by an increase of their sizes (swelling), after which the polymersomes start to shrink slowly, with pulsation episodes remaining active until the full system dries up. Figure 2 shows the initial size of a multivesicle cluster (A), the maximum size that the cluster reaches in the swelling process (B), and the final size, (C), after shrinkage has taken place.

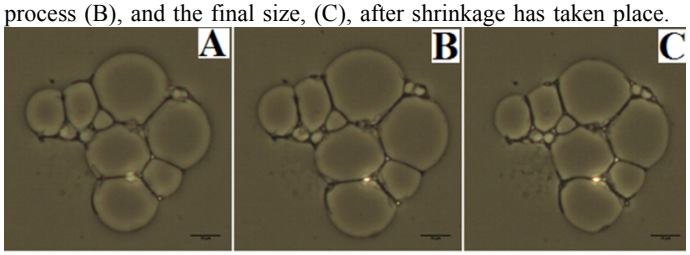

Figure 2: Polybutadiene-b-polyethylene oxide polymersome (PB46-b-PEO31) containing CHD-BZ reaction (H2SO4 600 mM, CHD 0.111 mM, NaBrO3 246 mM, Ferroin 3.5 mM) prepared using inverted method in sucrose solution 100 mM as imaged by phase contrast microscopy. The micrographs shown in panels A to C illustrate the time evolution of the vesicles clusters. The time and the percentage of swelling (+) or shrinkage (-) corresponding to each panel, and taking the initial size of the cluster as reference, are: A) 520 s (mean size 44μm); B) 1556s (+4.5 %) and C) 4260s (-8.2%). Scale bar 10 μm.

Second (PolBZ4.avi), polymersomes undergo pulsation episodes while shrinking, although now without any accompanying swelling processes. Figure 3 shows the temporal evolution of a typical cluster of vesicles undergoing this type shrinkage; in 3A, 3B and 3C we show the initial, intermediate and final states of the system as time elapses.

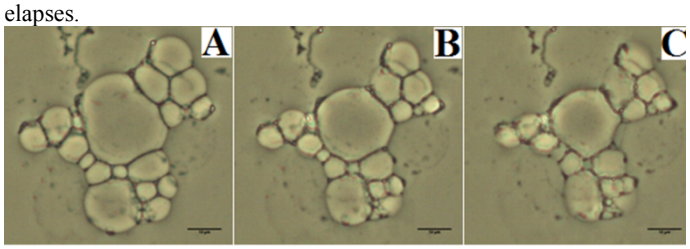

Figure 3: Polybutadiene-b-polyethylene oxide polymersome (PB_{46}-b-PEO_{31}) containing CHD-BZ reaction (H_2SO_4 600 mM, CHD 0.111 mM, $NaBrO_3$ 246 mM, Ferroin 3.5 mM) prepared using inverted method, in sucrose 100 mM. The phase contrast micrographs (Panels A to C) show the evolution of the cluster **shrinkage** process (no swelling was observed for these conditions). The sizes of the larger vesicle as well as the time are: A) (23 μm, 512 s); B) (20 μm, 2120 s) and C) (18 μm, 4120s). Scale bar 10 μm.

The above pulsation episodes also show very small membrane excursions. At first, this makes it difficult to quantify the effect. However, it demonstrates that the reaction is occurring only inside the vesicles (in a volume of appr. 10^{-14} m^3) and unveils the presence of small chemo-mechanical coupling effects. Interestingly, these are of the same order of magnitude as those reported in gels[22-24]. Since no membrane disruption was observed, we feel that in principle one can safely assume that the forces that keep the polymers assembled as bilayers are stronger then the chemo-mechanical forces induced by the CHD-BZ reaction inside the vesicle.

Conclusions

Forming PB-PEO polymersomes using the inverted emulsion method with the CHD-BZ reaction trapped inside the polymersome and with the polymersomes enclosed in a sucrose-enriched medium, we have been able to observe that oscillations in the BZ reaction get transmitted as vesicle oscillations. Therefore there exists chemo-mechanical coupling between the inner chemical oscillations and the containing vesicle. This form of chemo-mechanical coupling is, in principle, different from the well-known coupling that occurs in gels[22-25] or other bulk polymer systems where, unlike here the catalyst is cross linked to the polymer network. In our experiments the catalyst is dissolved in the BZ reaction media and our vesicle formation protocols do not involve any steps (for example exposure to UV light) that might have led to the crosslinkage of the catalyst used in chemically responsive gels. The coupling reported here takes place just at the boundary of the vesicle, and leads us to believe that it involves components of the chemistry, the mechanics of the vesicles, perhaps also including some entropic effects and the chemistry of the polymer per se. The study of this phenomenon and its analysis and impact for vesicle communication and quorum sensing phenomena, as well as a detailed characterization of all the environmental conditions will be carried out in a separate publication.

In summary we have demonstrated a) how to build low-pH resistant polymesomes, that b) can contain a low-pH redox oscillating chemical reaction, as is the case of BZ, c) that there exists oscillatory behavior in the vesicles and that d), this chemo-mechanical coupling is due to the chemical oscillation of the BZ catalyst. Our results will be useful, among other applications for the study of phenomena associated with the BZ reaction inside polymersomes. These phenomena for example include the coupling of BZ to the vesicle walls to implement valves, or to investigation of BZ-controlled vesicle fusion or collective properties of vesicle networks.

Notes and references

[a] Department of Earth and Planetary Sciences, Harvard University, Cambridge, Massachusetts 02138, USA
*tpereiradesouza@fas.harvard.edu

Electronic Supplementary Information (ESI) available: Miscellaneous Information.doc; PolW1.avi; PolBZ1.avi; PolBZ2.avi; and PolBZ3.avi See DOI: 10.1039/c000000x/

Acknowledgment: We thank Drs. Valentin Ruiz and Fernando Temprano for discussions and help in obtaining our polymers. This work was funded by Repsol, S.A. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Note†. Figures S4 and S5 in ESI show the stability of a polymersome containing a 600 mM sulphuric acid.

Note†† Sugars solutions are extensively used as enhancer for optical contrast in vesicle microscopy

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