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EFFECT OF THE TEMPERATURE ON THE CONTROLLED RELEASE OF ACTIVES IN GELLAN GUM-BASED MICROCAPSULES

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Abstract. *Microcapsules are composed of single or multiple cores, which can be made of gas bubbles or liquid drops, covered by a shell. They are attractive candidates for encapsulating, transporting, or controllably releasing a wide variety of important active materials. The delivery can be initiated by a wide variety of mechanisms, such as pH and temperature changes. In addition to being widely used in the pharmaceutical, food and cosmetic industries, microcapsules are also a viable solution in the biomedicine and in the petroleum industry. In times when sustainability is necessary to alleviate environmental issues, biodegradable substances become viable solutions for socio-environmental development. Thus, this study develops a technique for the controlled release of actives in thermosensitive microcapsules based on gellan gum biopolymer through a temperature trigger event. We studied the influence that different levels of temperature exert on the release time of the actives from the microcapsules. Tests were performed with both fixed and variable temperatures in order to investigate whether the heat rate influences the speed of the inner content's delivery. In addition, we also studied the effect that physical, such as shell diameter and shell thickness, have on the release rate. For this, the release dynamics of capsules with different diameters, from 190 to 500 μm, and shell thickness were studied. The results show how gellan gum microcapsules can be designed to control the release time of their inner content subjected to temperature changes.*

Keywords: *gellan gum, temperature, controlled release, microcapsule*

1. INTRODUCTION

Microcapsules are formed by a core material, single or multiple, involved by a membrane, also called shell (Datta *et al.*, 2014). This membrane act as an isolator to protect their cargo from the outer environment (Fang and Bhandari, 2010). They are often made from double, or single, emulsion droplets that later are transformed into capsules (Amstad, 2017) Several sectors can benefit from their applications as they are promising candidates for protection, transportation, delivery, and controllably release its core in targeted locations (Esser-Kahn *et al.*, 2011). Microcapsules have been used to encapsulate important technological actives (Dubey, 2009), including substances for enhanced oil recovery (Zhang *et al.*, 2020), agricultural odors to lure plague into traps, food additives, pharmaceuticals, and cosmetic components (Cheng *et al.*, 2008), cells (Uludag *et al.*, 2000), catalysts for chemical reactions (Pastine *et al.*, 2009), restorative agents for self-healing materials (Li *et al.*, 2020). These applications require an efficient shell that can retain and release its active only after a trigger stimulus (Sun *et al.*, 2010). The stimuli responsive microcapsules (Pentela *et al.*, 2019) have drawn attention worldwide because it can be conveniently used to the controlled release of molecules at a specific site and time by using an external stimuli such as temperature (Sun *et al.*, 2010) , light (Windbergs *et al.*, 2013), external stress (Leopércio, 2021), pH (Abbaspourrad *et al.*, 2013), and osmotic pressure. (Zhang *et al.*, 2017).

Environment regulations increase the demand for biodegradable components in the industry (Liserre *et al.*, 2007). Nowadays, using biopolymers is a promising innovation because reaches the most diverse fields with the extra benefit of its biodegradation capacity (Michelon *et al.*, 2020). In this work we use the biopolymer gellan gum, secreted by a microbial fermentation *Sphingomonas elodea*, as the shell substance of the microcapsule. These biodegradable-based microcapsules are available in two forms: soft, elastic, naturally high acyl form (HA) and a firm, brittle low acyl form (LA) (Mao *et al.*, 2000). The capsules were produced through a microfluidic approach using double emulsions templates in order to achieve tunable proprieties. The gellan gum gelation occurs though crosslinking induced by the presence of cations in the continuous phase during their production.

The controlled release of their inner content uses temperature as the trigger mechanism. The conditions to their

degradation are reported. We present how different microcapsules proprieties (sizes, shell thickness) respond to a steady and transit regime, i.e, fixing and varying the environment temperature, respectively.

2. GELLAN MICROCAPSULES

Based on Michelon *et al.* (2020) and Leopércio (2021) works, the production was made through a microfluidic capillary device, as shown in figure 1.A, using double emulsion templates, composed by sunflower oil in the inner and continuous phase. Gellan gum aqueous solution is used as the middle phase to form a bio-polymeric and elastic shell. In this work, t, is known as the thickness of the shell, while, D, is refered as the diameter of the microcapsule.

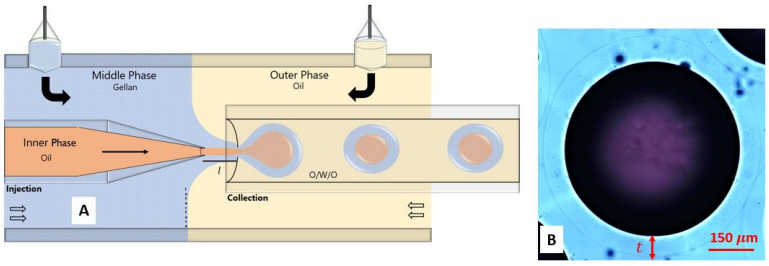

Figure 1. A) Detailed 2D diagram of the flow of liquids involved during the formation of double emulsions templates. B) Microcapsule produced.

The inner phase is a refined commercial sunflower oil (Liza, Cargill S.A, Brasil) with the addition of a purple or a yellow food-grade dye only for visualization purposes. The middle phase consists of a mixture of 0.5 wt% of low-acyl gellan gum Kelcogel CG-LA (CP Kelco Brasil S/A, Brazil) and 2 wt% of polyoxyethylene sorbitan monolaurete, Tween20 surfactant (Sigma-Aldrich, USA), in ultrapure water (Direct-Q3 UV System, Millipore Co., USA). The contiuous phase is a sunflower oil containing 1 wt% of calcium acetate (sigma-Aldrich, USA) and 5 wt% of polyglycerol-polyricinoleate emulsifier commercially named as Grinstead PGPR (Danisco, Brazil). The microcapsule produced is shown in figure 1.B.

The properties of the fluids used are summarized in the table 1 below. It is important to mention that the viscosity was measured in a shear rate window ranging from 10^{-1} to 10^{3} s^{-1} and the difference in the interfacial tension values between the internal-intermediate and intermediate-continuous phases is explained by the presence of tween20 and PGPR surfactants.

Table 1. Interfacial tension (σ), density (ρ) and viscosity (μ) of the respective phases of the low-acyl (LA) gellan microcapsules.

Phase	ρ [kg/m³]	μ [mPa.s]	σ [mN/m]
Continuous (O_O)	755.2 ± 0.1	55.3	5.4 ± 0.3
Middle (W)	1020.1 ± 0.9	2.2-100.7	
Inner (O_i)	752.8 ± 0.1	73.9	2.6 ± 0.2

In order to compare how physical characteristics affects the release time of the inner content, distinct samples were produced, as shown in table 2 below. Sample 1 was used to investigate the temperature trigger region, while samples 2 to 4 were used to evaluate the controlled release under different diameter and shell thickness proprieties and heating protocols. We produce capsules that are 200 to 400% larger than those made by de Lima *et al.* (2021) precisely to find out how different properties affect the degradation of them.

Table 2. Low-acyl microcapsules system produced with the microfluidic device.

Sample	Diameter [μm]	Thickness [μm]	t/D [%]
1	191.4 ± 2.2	11.5 ± 1.3	5.8
2	487.1 ± 42.8	50.4 ± 15.7	10.3
3	466.2 ± 30.9	34.2 ± 7.9	7.4
4	507.6 ± 27.3	21.9 ± 12.6	4.1

3. SETUP

The experimental setup used in this study is composed of a sealed support for the capsules, a heater, an inverted microscope, a controlled temperature bath, and a computer to acquire and process the images, as shown in figure 2.

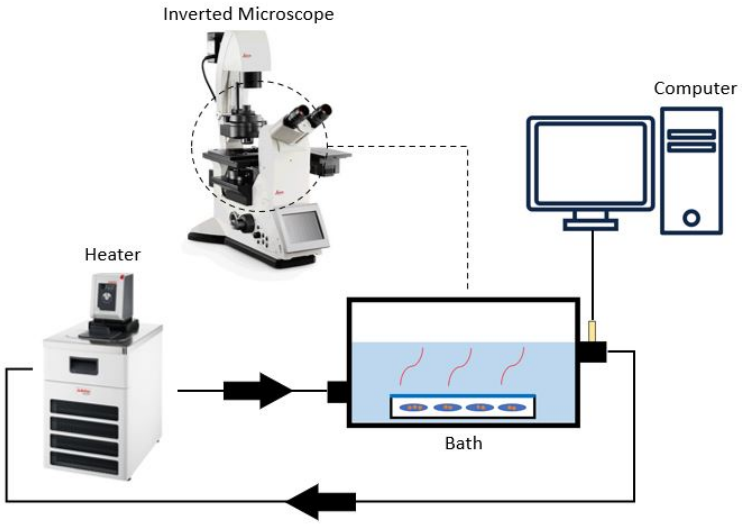

Figure 2. Experimental setup used to evaluate the controlled release under temperature trigger stimuli.

A kline's plate glass (10x7x2 cm, Qualividros Distribuidora LTDA) with twelve several excavations of 1 ml each was used as a support to separate each sample of capsules. Thus, the microcapsules are isolated from direct contact with the heating fluid. The sealing system, besides the kline's plate, is composed by the addition of grease on the surface of the support and silicone glue on its sides.

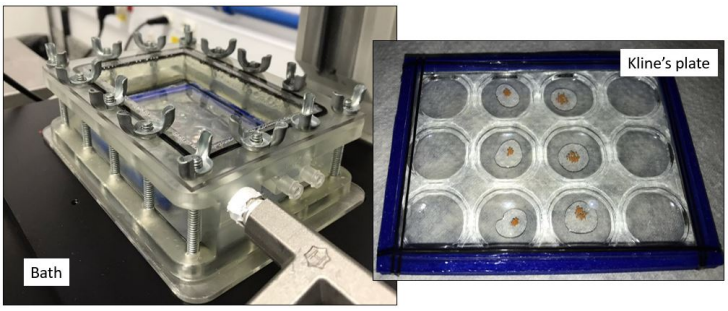

Figure 3. The printed bath on the left and, on the right, the kline's plate.

The bath, as shown in figure 3, was designed with the software Solidworks®, printed by a 3D printer (model SLA, Formlabs, USA) and an acrylic plate was designed and ordered to fit between the support and the bath. Temperature was measured by a thermocouple (National Instruments, USA, Model DR-4524) and the LabView®software was responsible for read and register the data. Finally, an inverted microscope (model DMi8, Leica Microsystem, Germany) was used for all visual part of the experiments.

The process for this controlled release basically drains deionized water (heating fluid) through the bath. Inside it, we find the microcapsules sealed in the kline's plate (figure 3, right). As the heating fluid reaches a certain height, it flows back to the heater, initiating the circulation of the test. Meanwhile, we analyze the behavior of these microcapsules with Leica's microscope .

4. HEATING PROTOCOLS

The tests were divided between two heating protocols. Among them are fixed and variable temperature tests.

- **Gradual:** Tests were carried out to find the ideal region at which the shell reacts to the stimuli of the environment. For this type of protocol, the experiment started at room temperature and ended at the temperature of reaction to the thermal trigger. In addition, this protocol was also used to evaluate how the heat rate increase can influence the release speed of the inner contents of the microcapsule.

- **Fixed temperature:** This protocol set the working temperature at 75C, the optimum temperature found by de Lima *et al.* (2021), and served as a basis for evaluating the evolution of degradation for different proprieties of the microcapsules.

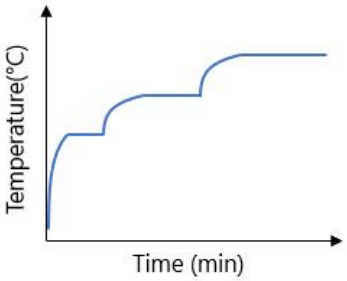

Figure 4. Gradual increase protocol

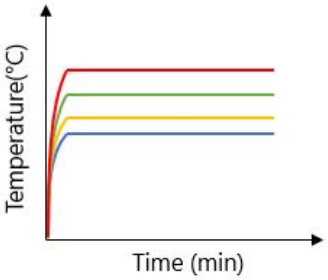

Figure 5. Fixed temperature protocol which each color represent a different test.

5. RESULTS AND DISCUSSION

5.1 TRIGGER TEMPERATURE REGION

In order to define the ideal temperature region at which the shell reacts to the temperature stimuli, we used the first protocol of gradual temperature increase. The test was focused on the capsule shell, without any type of quantification, just observing in detail the shell's reaction to the stimulus and thus finding the temperature region at which it is most sensitive dispersed in acetate buffer (ph=4.5).

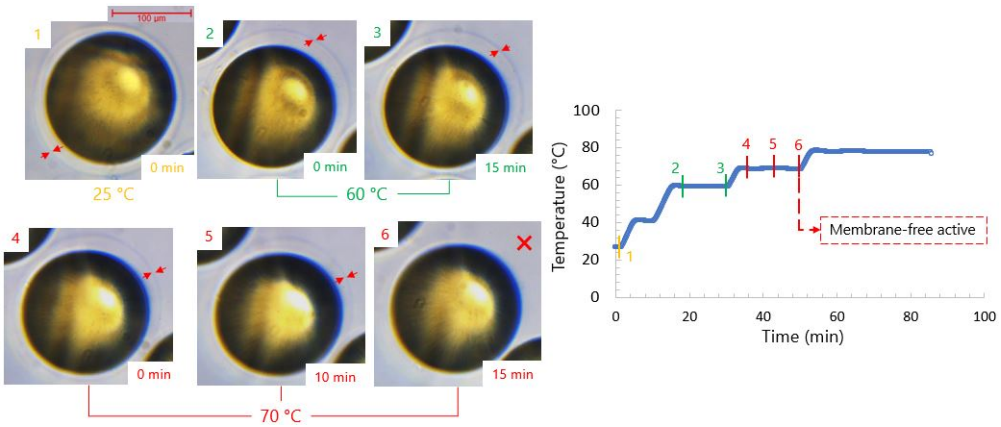

Figure 6. Gradual increase test; (1) Original capsule at room temperatuere. (2-3) At 60°C the membrane is still intact with few modification in its eccentricity. (4-5) 10 minutes at 70°C with the shell still observed. (6) After 15 min, the shell wall no longer exists. Red arrows represent the contour of the shell whil the "x" indicates that the shell is no longer visible.

The 20x zoom lens of the Leica® system provided the visualization focused only on the behavior of the capsule's membrane. As figure 6 shows, the first changes starts at 60 °C (2), changing the capsule's eccentricity. Increasing the temperature level to 70 °C it is observed that, within 15 minutes, the membrane no longer exists (6). The shell disappearance should be an indicator that the trigger for its degradation is in the range of 70 °C. This temperature region of degradation corroborates with de Lima *et al.* (2021) work.

5.2 FIXED TEMPERATURE

We determined the optimal working temperature at which all gellan microcapsules reacts to the stimulus at 75 °C. W set this temperature level in our tests in order to evaluate the evolution of the release for different sizes and thicknesses of

the microcapsule's shell.

Figure 7 shows the evolution of the number of remaining capsules within a 90 minutes window time test. We noticed that the first big difference is in the degradation time. The green arrows represent the degradation region reached by the de Lima *et al.* (2021), with a maximum elapsed time of 10 minutes. However, the significant time increase in this work can be explained by the size difference between them. While the largest microcapsules of the de Lima's had 220 μm, the smallest diameter worked for this kind of test, in this work, is approximately 466 μm.

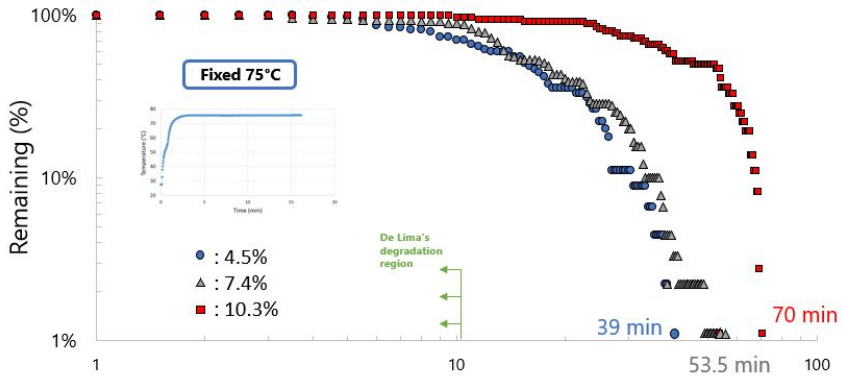

Figure 7. Evolution of the number of remaining large microcapsules system within 90 minutes operability window for several thickness steps under the fixed temperature protocol.

We can see the difference that each level of thickness exerts on the response to the trigger. Samples 3, in dark blue (D=507 μm, t/D=4.1%), and 2, in gray (D=466.2 um μm, t/D=7.4%), despite having a similar behavior, they have different times at the end of the degradation of 39 and 53.5 minutes, respectively. Sample 1, in red (D=487.1 μm, t/D=10.3%), shows the biggest difference within the system. In addition to having a slower stimulus response, it also has the longest time (70 minutes) required to deliver all loaded actives compared with the other two samples.

Unlike de Lima's work, larger and thicker capsules have a greater tendency towards temperature resistance. This could be explained by the greater mass present both in the total particle diameter (internal phase plus shell) and the membrane alone. That is, the thicker the shell is, the longer time/heat will be required to completely degrade the microcapsules.

5.3 VARIABLE TEMPERATURE

One way to try to accelerate the release time is to change the outer environment temperature. We study the dynamics of the same system as the temperature was increased above 75 °C. So, the temperature control method changed from fixed temperature to gradual increase protocol. The idea was to start at the temperature (75°C), based on de Lima *et al.* (2021), and increase it after a pre-setted period. We selected the increases to 85°C at 15 minutes and 90°C at 20 minutes.

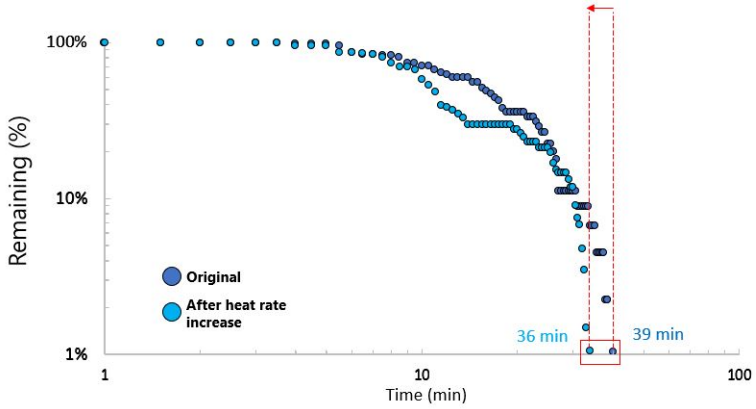

Figure 8. Comparison of the degradation evolution of thin shell microcapsules from sample 4 (D=507 μm, t/D=4.5%) between the temperature increase (light blue) and fixed temperature (blue) protocols.

Figure 8 compares the lowest thickness ratio batch (D=507.6,t/D=4.5%) where the degradation evolution is similar at the beginning and in the middle of the test. However, the total release time for all assets has decreased, reducing it from 39 minutes (original) to 36 minutes. Although it only reduced the total time by 7.69%, it is an indication that the temperature increase influenced the temperature trigger response.

The investigation continued for the system sample with the intermediate t/D ratio (D=466.2,t/D=7.4%), as shown in figure 9, and we concluded that the behavior throughout the test is similar. However, there is a faster reaction at the end compared to the original, demonstrating once again the heat rate influence to the system, accelerating the delivery. There is a 31.80% reduction in degradation timeincreasing the heat rate. While the original test took 53.5 minutes, the altered took 36.5 minutes. In this case, we already see a greater percentage of reduction in comparison to the first sample.

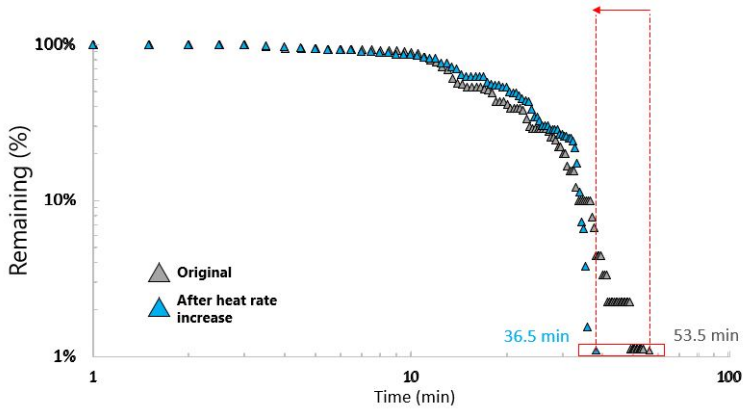

Figure 9. Comparison of the degradation evolution, from sample 3, with thicknes/diameter ratio of 7.4% (D=466.2 μm) between the temperature increase (light blue) and fixed temperature (gray) protocols.

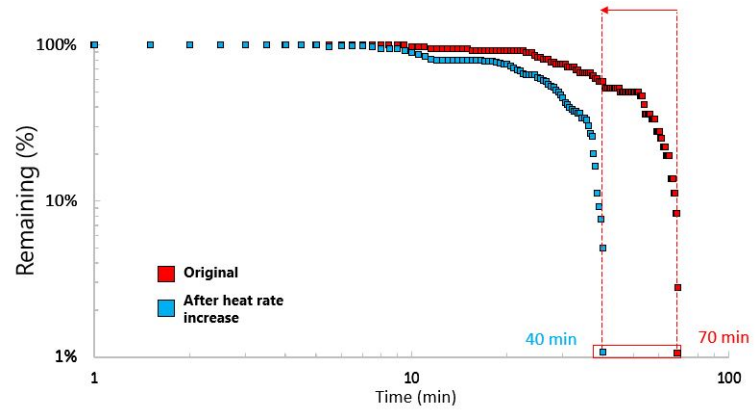

Figure 10. Comparison of the degradation evolution of the thicker shell microcapsules, from sample 2 (D=487 μm, t/D=10.3%) between the temperature increase (light blue) and fixed temperature (red) protocols.

Finally, as shown in figure 10, we ended up with the test of the greater thickness of the system (D=487.1,t/D=10.3%). Unlike the other two cases, the behavior was shifted and modified from start to finish, showing a greater sensitivity of response to the higher imposed temperature levels, shown in figure 10. While the original test lasted about 70 minutes, the modified one reduced to 40 minutes. This half-hour decrease represents a 42.9% shorter time when working with higher temperatures concerning its optimal degradation temperature.

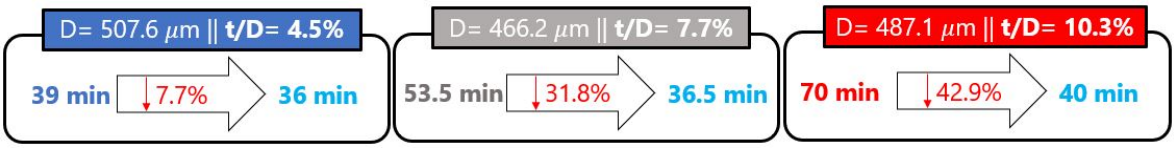

Figure 11. Evolution of the reduction in the time required to release the entire contents of the microcapsules for different shell thicknesses.

Figure 11 compares the difference between the fixed and variable temperature tests. It is noticed that, as we increase the thickness of the shell, the percentage of reduction of time elapsed increases.

6. CONCLUSION

This work showed that gellan-based microcapsules can be used to controlled release of their inner content by a temperature change trigger. The time of delivery can be controlled by the geometry of the capsules and material used in the shell. The stimuli response was analyzed at different temperature levels to find an optimal test temperature. The results show that the size and the thickness-diameter ratio matter on the release dynamics. Microcapsules with the lowest t/D ratio ($t/D = 4.5\%$) requires 27.1% less time than sample 3 ($t/D = 7.7\%$) and 44.3% less if we compare with sample 2 ($t/D = 10.3\%$). Even if we compare the intermediate t/D ratio ($t/D = 7.7\%$) with the largest ratio ($t/D = 10.3\%$), the same logic repeats, and the degradation is 23.6% faster.

In order to accelerate the release, the heat rate increase protocol was implemented. The fixed temperature protocol (original) was slower comparing with the heat rate increase protocol. Thus, the elapsed time has been greatly reduced. If we compare each microcapsules sample for each kind, the results also show a faster response with new stimuli. For sample 4 ($D = 507.6\ \mu m$, $t/D = 4.5\%$) had a 7.7% reduction percentage comparing with the original. While sample 3 ($D = 466.2\ \mu m$,$t/D = 7.7\%$) and 2 ($D = 487.1\ \mu m$,$t/D = 10.1\%$) reduced its time elapsed in 31.8% and 42.9%, respectively. The percentage of time reduction increased as the shell thickness increased. Higher amounts of gellan in the shell were shown to be more sensitive to the temperature trigger.

To continue this research in future works, some suggestions may be considered as studying the addition of the high acyl (HA) gellan chain in the microcapsules shell in order to evaluate how it can influence degradation.

7. ACKNOWLEDGEMENTS

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8. REFERENCES

Abbaspourrad, A., Datta, S.S. and Weitz, D.A., 2013. "Controlling release from ph-responsive microcapsules". *Langmuir*, Vol. 29, No. 41, pp. 12697–12702.

Amstad, E., 2017. "Capsules: their past and opportunities for their future".

Cheng, S., Yuen, C., Kan, C.W. and Cheuk, K., 2008. "Development of cosmetic textiles using microencapsulation technology". *Research Journal of Textile and Apparel*.

Datta, S.S., Abbaspourrad, A., Amstad, E., Fan, J., Kim, S.H., Romanowsky, M., Shum, H.C., Sun, B., Utada, A.S., Windbergs, M. *et al.*, 2014. "25th anniversary article: Double emulsion templated solid microcapsules: Mechanics and controlled release". *Advanced Materials*, Vol. 26, No. 14, pp. 2205–2218.

de Lima, M.A.R., Avendaño, J.A. and Carvalho, M.S., 2021. "Controlled release of the inner content of gellan gum microcapsule using temperature as the trigger mechanism". *Cobem 2021*.

Dubey, R., 2009. "Microencapsulation technology and applications". *Defence Science Journal*, Vol. 59, No. 1, p. 82.

Esser-Kahn, A.P., Odom, S.A., Sottos, N.R., White, S.R. and Moore, J.S., 2011. "Triggered release from polymer capsules". *Macromolecules*, Vol. 44, No. 14, pp. 5539–5553.

Fang, Z. and Bhandari, B., 2010. "Encapsulation of polyphenols–a review". *Trends in Food Science & Technology*, Vol. 21, No. 10, pp. 510–523.

Leopércio, B.C., 2021. *Gellan-based microcapsules: production and applications*. Ph.D. thesis, PUC-Rio.

Li, J., Yang, S., Muhammad, Y., Sahibzada, M., Zhu, Z., Liu, T. and Liao, S., 2020. "Fabrication and application of polyurea formaldehyde-bioasphalt microcapsules as a secondary modifier for the preparation of high self-healing rate sbs modified asphalt". *Construction and Building Materials*, Vol. 246, p. 118452.

Liserre, A.M., Ré, M.I. and Franco, B.D., 2007. "Microencapsulation of bifidobacterium animalis subsp. lactis in modified alginate-chitosan beads and evaluation of survival in simulated gastrointestinal conditions". *Food Biotechnology*, Vol. 21, No. 1, pp. 1–16.

Mao, R., Tang, J. and Swanson, B., 2000. "Texture properties of high and low acyl mixed gellan gels". *Carbohydrate polymers*, Vol. 41, No. 4, pp. 331–338.

Michelon, M., Leopércio, B.C. and Carvalho, M.S., 2020. "Microfluidic production of aqueous suspensions of gellan-based microcapsules containing hydrophobic compounds". *Chemical Engineering Science*, Vol. 211, p. 115314.

Pastine, S.J., Okawa, D., Zettl, A. and Fréchet, J.M., 2009. "Chemicals on demand with phototriggerable microcapsules". *Journal of the American Chemical Society*, Vol. 131, No. 38, pp. 13586–13587.

Pentela, N., Rainu, S., Duraipandy, N., Boopathi, A., Kiran, M., Sampath, S. and Samanta, D., 2019. "Microcapsules responsive to ph and temperature: synthesis, encapsulation and release study". *SN Applied Sciences*, Vol. 1, No. 5, pp. 1–10.

Sun, B.J., Shum, H.C., Holtze, C. and Weitz, D.A., 2010. "Microfluidic melt emulsification for encapsulation and release of actives". *ACS applied materials & interfaces*, Vol. 2, No. 12, pp. 3411–3416.

Uludag, H., De Vos, P. and Tresco, P.A., 2000. "Technology of mammalian cell encapsulation". *Advanced drug delivery*

reviews, Vol. 42, No. 1-2, pp. 29–64.

Windbergs, M., Zhao, Y., Heyman, J. and Weitz, D.A., 2013. "Biodegradable core–shell carriers for simultaneous encapsulation of synergistic actives". *Journal of the American Chemical Society*, Vol. 135, No. 21, pp. 7933–7937.

Zhang, L., Abbaspourrad, A., Parsa, S., Tang, J., Cassiola, F., Zhang, M., Tian, S., Dai, C., Xiao, L. and Weitz, D.A., 2020. "Core–shell nanohydrogels with programmable swelling for conformance control in porous media". *ACS Applied Materials & Interfaces*, Vol. 12, No. 30, pp. 34217–34225.

Zhang, W., Abbaspourrad, A., Chen, D., Campbell, E., Zhao, H., Li, Y., Li, Q. and Weitz, D.A., 2017. "Osmotic pressure triggered rapid release of encapsulated enzymes with enhanced activity". *Advanced Functional Materials*, Vol. 27, No. 29, p. 1700975.

9. RESPONSIBILITY NOTICE

Mateus Aguiar Rodrigues de Lima, Jorge Avendaño Benavides and Marcio da Silveira Carvalho are solely responsible for the printed material included in this paper.