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CONTROLLED RELEASE OF THE INNER CONTENT OF GELLAN GUM MICROCAPSULES USING TEMPERATURE AS THE TRIGGER MECHANISM

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Abstract. *Microcapsules are formed by a core material involved by a membrane. They have an important role in food and pharmaceutical industries due to their capacity to improve ingredient handling, to mask undesirable flavors, odors and to protect different components from chemical or physical degradation during storage or after ingestion, controlling the release of the encapsulated ingredient. The use can be extended to different applications that require the protection of the inner phase and its controlled release. Different triggers can be applied in order to activate the core release, depending of the application and capsule material. In the present work, we study the controlled release of the inner content of a bio-polymer base microcapsule, using temperature as the trigger mechanism. We analyze how the temperature and capsule properties (material, diameter and shell thickness) affect the release time. The release of the inner content is determined by adding a dye in the inner phase of the capsules and observing a suspension of microcapsules inside a controlled temperature bath in an inverted microscope. The results presented in this work show how different capsules can be design to deliver its inner content at different levels of temperature and residence time, we report two different approaches on rising the temperature of our particles and a promising trigger condition in order to disintegrate its shell.*

Keywords: *microcapsules, controlled released, microfluidics*

1. INTRODUCTION

A microcapsule is a micro-meter scale particle, bubble or a liquid drop that is surrounded by a membrane (Datta *et. al*, 2014). This membrane act as an isolator to protect their cargo from the outer environment. Microcapsules are applied in several sectors, and they have been used to encapsulate essential oils, anti-aging cosmetic products, sweeteners, and vitamins in the food industry. Also, they allow means to cure or reduce side-effects in a living organism and are also capable of incorporate properties that could self-heal structures. Thus, they are attractive candidates for encapsulating and transporting important active materials (Amstad *et al.*, 2017; Abaspourrad *et al.*, 2013; Werner *et al.* 2018). These kinds of applications require an efficient membrane/shell that can retain and release its active only after a specific stimulus (Sun et al. 2010). The release is a consequence of a reaction between the environment and the membrane leading to melting, diffusion or breaking its structure. The specific stimulus is related to the external conditions at which the capsules are involved in, such as: pH, temperature, osmotic pressure, UV lights, stress, or chemical reaction (Datta *et al*., 2014). The use of biodegradable polymers as the membrane material broadens the range of applications to areas at which requires the relation between living organisms and natural degradation of capsules (Michelon *et al*. 2020).

Biopolymer-based microcapsules contain a biopolymer as the shell phase and, in general, are obtained after a solidification of the middle phase of a double emulsion template. Microfluidic approach offers a route to overcome the poorly controlled encapsulation (Chen *et al*. 2012; Zhao *et al*. 2011; Michelon *et al*. 2019, do Nascimento *et al.* 2017, Yamamoto *et al.* 2007) and release characteristics of the conventional methods, emerging as a promising technique to produce monodispersed microcapsules with well-controlled release characteristics (Sun *et al.*, 2010).

The gellan gum is a polysaccharide produced by a microbial fermentation *Sphingomonas elodea* and is commonly used as thickener and stabilizer particle in the food processing industry. It is available in two forms: soft, elastic, naturally high acyl form (HA) and a firm, brittle low acyl form (LA). The degradation of polysaccharides has been analyzed as a function of some processes, such as temperature (Fisher *et al.*, 2011; Mao *et al*. 2000; Bacelar *et al*. 2016; Graham *et al*. 2019).

In this work, we study the response of gellan gum microcapsules to changes in temperature. The results show how different capsules can be designed to deliver its inner content at different levels of temperature and residence time.

2. SETUP AND MATERIALS

2.1. Microcapsules.

The monodispersed microcapsules were produced by microfluidics from O/W/O templates (Costa *et al.* 2017) in which the devices were built on a glass slide and consisted of two cylindrical glass-capillaries mounted inside a square capillary (Michelon *et al.* 2020). The biopolymer-based middle phase consisted of a mixture of 0.5 *wt%* low-acyl (LA) gellan gum Kelcogel_ CG-LA (CP Kelco Brasil S/A, Brazil) and 2 *wt%* polyoxyethylene sorbitan monolaurate, $Tween^{®}$ 20 (Sigma-Aldrich, USA), in ultrapure water with resistivity 18 $M\Omega/\mathrm{cm}$ (Direct-Q3 UV System, Millipore Co., USA) and was previously mixed at $80\,°C$ for 10 min under magnetic stirring. The inner phase was a commercial sunflower oil (Liza, Cargill Agricola S.A., Brazil). The continuous phase was a sunflower oil dispersion containing 1 wt% calcium acetate (Sigma-Aldrich, USA) and 5 wt% polyglycerol-polyricinoleate commercially named Grinstead® PGPR super (Danisco Brasil, Brazil). The properties of the liquid phases used to produce the microcapsules are presented in Table 1.

Table 1. Interfacial tension ($\boldsymbol{\sigma}$), density ($\boldsymbol{\rho}$) and viscosity ($\boldsymbol{\eta}$) of the respective phases of the gellan microcapsules.

Phase	**Composition**	**ρ (kg/m³)**	**σ (*mN/m*)**	**η (*mPa.s*)**
Inner (Oi)	Sunflower oil	752.8 ± 0.1	5.4 ± 0.3	55.3
Middle (W)	Ultrapure water Gellan gum (0.5 *wt%*)	1019.1 ± 0.9		21.6-291.4
Continuous (Oo)	Sunflower oil PGPR (5 *wt%*) Calcium acetate (1 *wt%*)	755.2 ± 0.2	2.7 ± 0.2	73.9

After production, the capsules were collected in a vial with hexane and acetate buffer solution (0.074 *mol/L*, pH 4.5) was added to disperse the produced microcapsules in an aqueous phase. The hexane excess containing the oil from the continuous phase was removed and the residual hexane was evaporated at room temperature during 24 h, turning the initial O/W/O template to a W/W/O solution.

2.2. Experimental Setup

In order to study the behavior of the microcapsules at different levels of temperature, an experimental setup and procedure was developed, as shown in Figure 1.

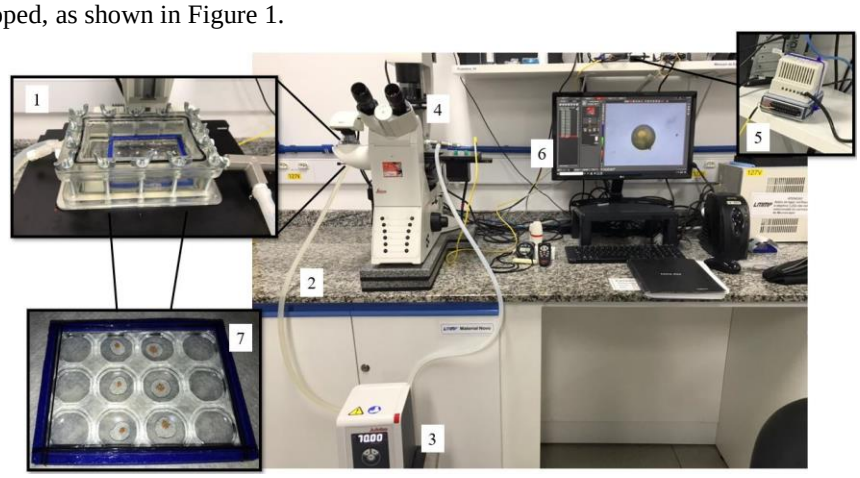

Figure 1. Experimental setup for the controlled release of gellan capsules.

The setup consisted of a liquid bath (1), hoses (2), heater (3), an inverted microscope (4), a thermostat (5), computer (6), thermometer and finally the microcapsules holding device (7). The bath was built with a 3D printer (model SLA, Formlabs, USA) measuring 7 cm of height, length of 16 cm and 10.5 cm of depth. These measurements are not random, they were precisely designed to fit the inverted microscope (model DMi8, Leica Microsystem, Germany) and the support of the capsules inside the bath.

The capsules holding device (Kline's glass plate, 6x8 *cm*) consisted of small basins, where each one of them contained capsules with different properties. The inverted microscope was used to measure the evolution of the outer diameter (D) and the shell thickness (t) of the microcapsules at different temperatures. In order to seal the compartment, we used a glass cover (100 mm x 70 mm) with a printed support to fit the device. For the sealing, materials capable of withstand

high temperatures were chosen such as grease, silicone, and O-ring. In addition, we fitted another O-ring at the bottom of the bath to prevent the possibility of leakage of the heating fluid (deionized water). The heater (model CORIO CD, Julabo GmbH, Germany) was responsible for store, heat and flow water through the bath.

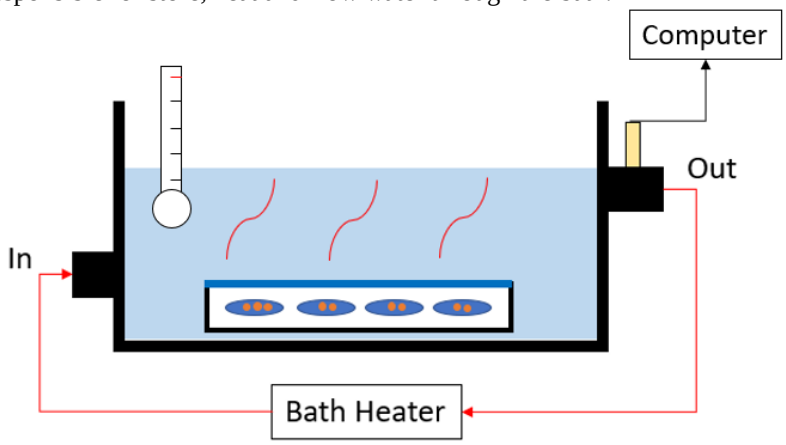

Figure 2. Schematic representation of the bath heating process.

Finally, the thermostat provided temperature data in real time, in addition we had a thermometer to compare both temperature measurements. The layout of this process is shown in figure 2. So basically, the hot water (heating fluid) flows through the hose draining the bath. Inside it, we find the microcapsules sealed in the kline's plate. As the heating fluid reaches a certain height, it flows through a second hose back to the heater, initiating the circulation of the test. Meanwhile, we analyze the behavior of these microcapsules from the inverted microscope data.

2.3. Temperature Mechanism

We study the capsule evolution when submitted to a gradual increase in temperature and to a step change. In the gradual temperature rise experiments, the temperature started at 25 °*C* and ended at 75 °*C*. In the step change experiments, the final temperature varied between 70 °*C* to 75°*C*. In each experiment, we quantified the number of capsules that degraded over time. We also run single-capsules test to analyze the degradation process in more detail.

3. PARTIAL RESULTS

The results presented in this section show how different capsule properties can affect the degradation time. The idea is to compare the behavior for different thicknesses and diameter of microcapsules under the same temperature trigger conditions.

3.1. Aging comparison

The first tests were performed to evaluate how the aging time of the microcapsules affect the cargo release. They were performed using capsules produced more than one year ago and freshly produced capsules of the same properties. Experiments were performed varying the external environment temperature from 25 °C to 75 °C. Properties of the older and the recent microcapsules are shown in table 2.

Table 2. Properties of the older and recently produced microcapsules.

Condition	Thickness (t) [μm]	External Diameter (D) [μm]	Ratio (t/D)	Bulk pH
Old (#1)	11.4± 2.2	182.52 ± 5.22	0.062	4.5
New (#2)	11.5± 1.3	191.44 ± 2.18	0.058	4.5

The membrane's behavior during the heating process is shown in Figure 3. Once the membrane degrades, the oil cargo does not mix with the continuous phase due to their immiscibility (W/O) and that is why, in our case, the oil spherical shape it is not easily broken. Only when the thermal energy is capable of surpass the interfacial tension, we have the formation of an oil film heading to the surface of the water phase.

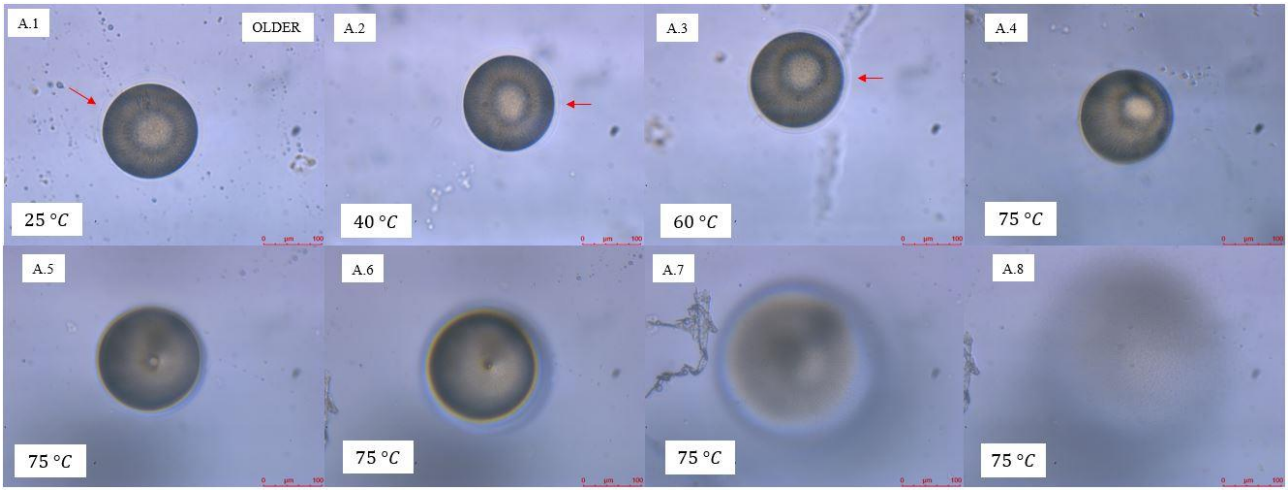

Figure 3. Temperature trigger event 25°C to 75°C: A.1) Unaltered microcapsule at the beginning of the test; A.2-A.3) Increase in temperature did not change its structure; A.4) Unseen shell; A.5-A.8) Oil film formation after losing its shell

At the beginning of the test (A.1) there is a membrane involving the sunflower oil cargo for the microcapsules at 25 °C. This structure does not change through 40°C (A.2) and 60°C (A.3). When the temperature reaches 75°C (A.4), 10 minutes after the beginning of the test, we can observe the first major changes in the membrane structure. The previous evident membrane does not exist, and we can clearly see a visual texture change on the microcapsule surface (A.5). After 15 minutes at 75°C (A.5 - A.8) there is an oil film formation. For most of the recent produced capsules trigger test, we could indeed see the membranes disintegration after the equivalent amount of test time.

So, for the aging comparison test we did not have major differences between the older and newer gellan shell, and the controlled release of the inner capsules content was not affected by the microcapsules production date.

3.2. Effect of final temperature

We quantify the inner phase release and study the effect of the final temperature on the step change temperature experiments by counting the number of remaining capsules as a function of time and temperature set. The results are presented in Figure 4. Final temperature below 70 °C did not show any capsule rupture.

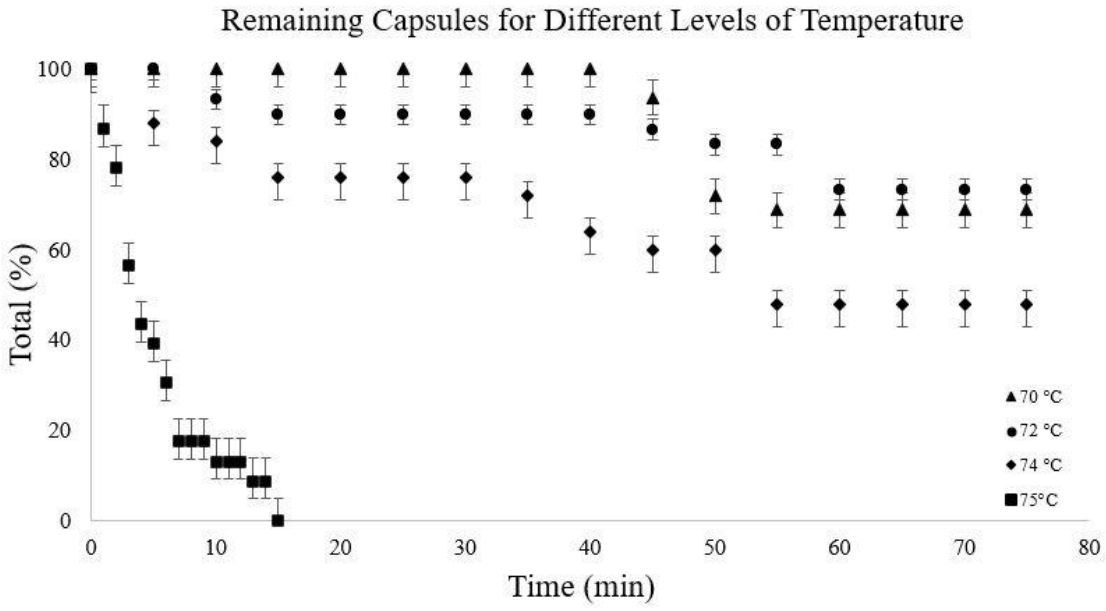

Figure 4. Temperature nfluence on the percentage of remaining capsules for different temperature levels.

The batch analyze had 191.44 $\pm$ 2.18 μm of diameter (D) and a shell thickness (t) of 11.5 $\pm$1.3 μm , resulting in a ratio (t/D) of 0.058. As we can see in Figure 4, at 70°C, a small number of capsules are destroyed. On the other hand, 75°C proved to be more viable for the contents total release. We can see an abrupt drop in the number of remaining capsules, acting as an instant stimulus to this temperature level leading to a total degradation. One of the main reasons that could explain this phenomenon is the polymer hydrolysis (Padsalgikar, 2017) in which at optimum trigger temperature, that we are trying to reach, under acidic conditions produces monosaccharides by breaking the glyosidic links between the monomer units in the structure of the molecule. Our continuous phase is acetate buffer (pH = 4.5) that was used in order to stabilize our microcapsules. Thus, as our particles are inserted under these acidic condition and are involved with this heating stimulant, hydrolysis seems to be a promising consequence for this kind of release mechanism.

3.3. Effect of microcapsule dimensions

As mentioned earlier, we ran test under variable and constant temperature. For both of them 75°C was the optimum trigger temperature observed during this study. In this subsection we analyze the diameter size and the thickness-diameter ratio influence on the release time under these different procedures for the trigger release study.

3.3.1 Condition 1: Variable temperature (25 °C to 75 °C)

In this condition, we worked with two microcapsule batches and their properties are presented in table 3 where the idea was to differentiate time response. The capsules have a similar t/D ratio. The batch MAT#36 has capsules 20% smaller than MAT#28.

Table 3. Batches with different diameter sizes

Batch	Thickness (t) [μm]	Diameter (D) [μm]	Ratio (t/D)	pH (bulk)
MAT #28	11.5$\pm$ 1.3	191.44 ± 2.18	0.058	4.5
MAT #36	10.1$\pm$ 1.4	169.76 ± 3.17	0.059	4.5

For this test we worked with the changing temperature procedure, starting at 25 °C and reaching 75 °C. Figure 5 presents the area of the circle defined by each capsule as a function of time. The final temperature of 75 °C was reached after approximately 8 minutes. Capsules from the MAT#28 batch presented a faster response. The external diameter (D) of the capsules decreases at 10 minutes (2 minutes after reaching 75 °C), which is associated with the degradation of the shell. At 15 minutes, the diameter rises, which corresponds to the formation of an oil film on the top of the continuous phase. The response of the MAT#36 batch was similar, but it took longer to be observed. The degradation of the shell was observed at 22 minutes and the formation of the oil film was observed approximately at 35 minutes.

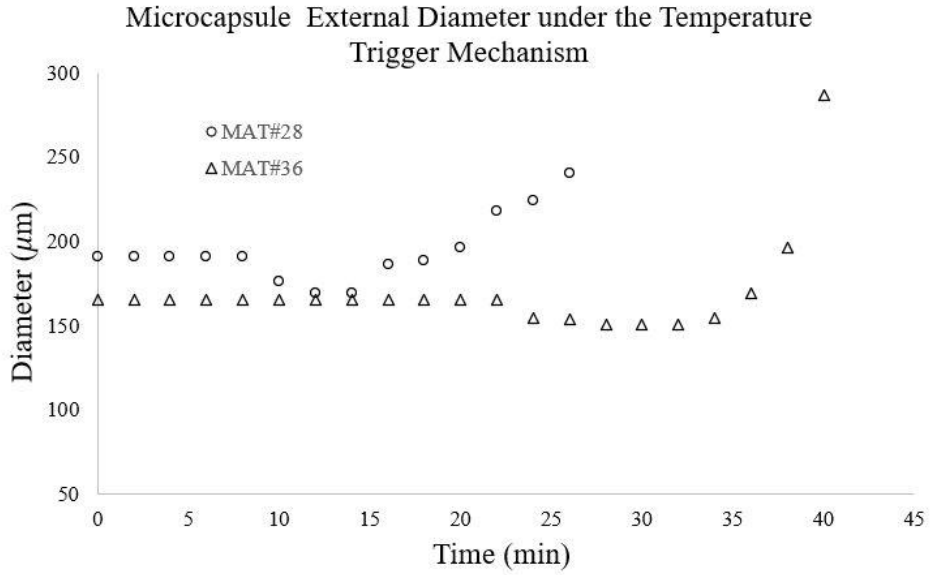

Figure 5. Schematic diagram of the control strategy.

The difference between the capsules was time response. In batch MAT#36, we can clearly see a late trigger response compared to the MAT#28, as the degradation began around 15 minutes after stabilizing at 75 °C. Figure 6 presents snap-shots of the degradation process for the MAT#36 capsules.

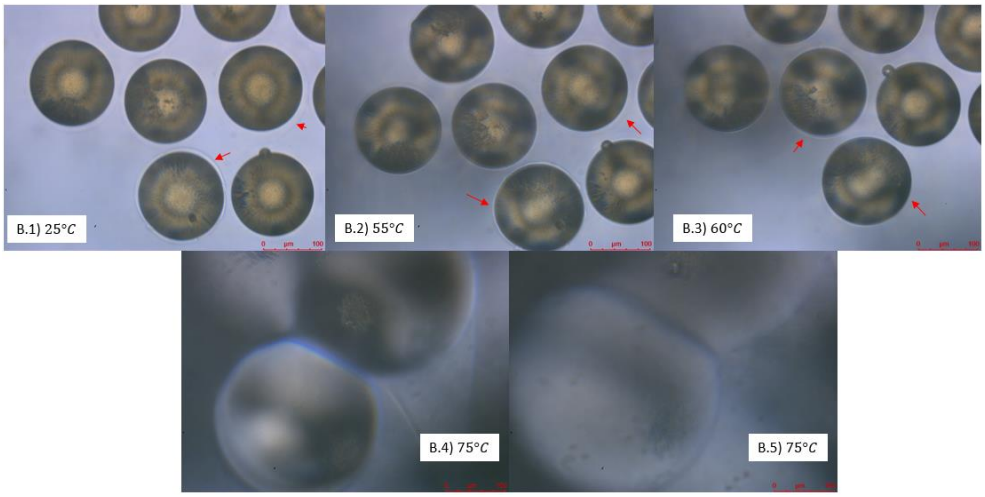

Figure 6. Coalescence example when there are capsules together; B.1-B.3: The membrane is still visible; B.4-B.5: The inner content was released after shell degradation.

3.3.2 Condition 2: Constant temperature

As shown in section 3.2, the most successful temperature involved in our experiments that could trigger a full cargo release was 75°C and, therefore, we analyze the degradation event under this constant temperature. For that, we divided the experiment into two different diameter groups for the microcapsule and, for each one of them, we aimed the same t/D ratio for the samples. For the first one, the diameter of the samples have an average value of 220 μm while the second have 185 μm. The groups are shown in table 4.

Table 4. Groups separated by size and an equivalent t/D ratio values

Group 1 (D = 220μm)		**Group 2 (D = 185 μm)**	
Sample	**Ratio t/D**	**Sample**	**Ratio t/D**
#1	0.025	#5	0.022
#2	0.036	#6	0.032
#3	0.045	#7	0.055
#4	0.057	#8	0.058

The quantification of the percentage number of the remaining intact capsules throughout the tests were based on frame analysis of the recorded videos and in each sample the average amount of microcapsules was 30 particles per test. In order to distinguish the intact and the ruptured ones, we observed difference in the structure of the particles where a clear alteration on our field of view is presented as well as a likely possible hydrolysis reaction (see section 3.2). Figure 7 shows a detailed structure alteration while figure 8 represents an example from one of the samples in real time with a broader line of view of the experiment.

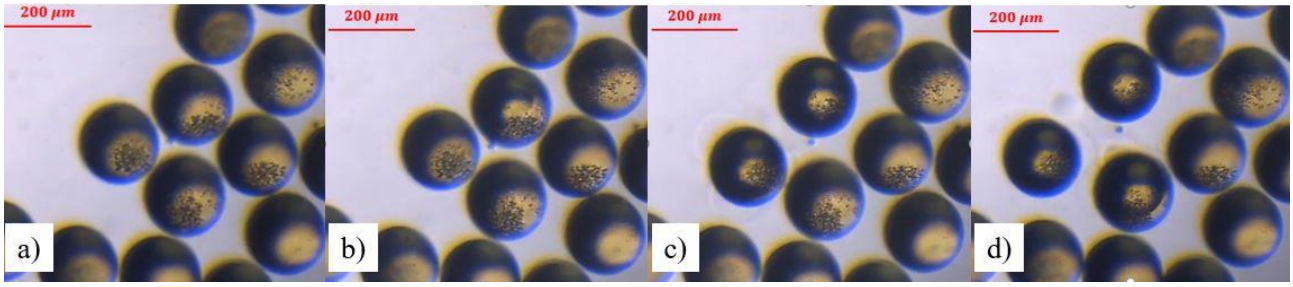

Figure 7. Detailed temperature trigger event: a) First minutes of the test at 75 °C with an unaltered microcapsule structure; b) Start of structure change; c-d) Two more shell destruction, only remaining the unprotected oil cargo.

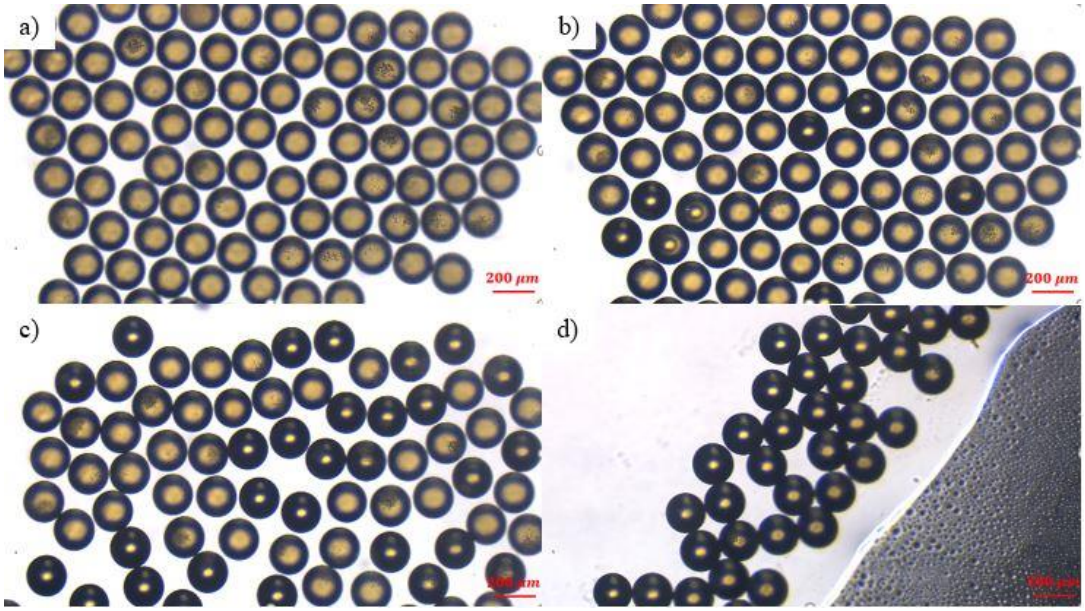

Figure 8. Quantification experiment at 75°C: a) Unaltered capsules in the beginning of the test; b) Trigger reached within 6 min and 20 s; c) Trigger chain reaction; d) Full contents release followed by oil coalescence.

After all data analysis, we registered few differences between the two groups. For the first one, the particles with the smallest t/D ratio (0.025) had a rougher response, in the percentage of the remaining capsules, to the temperature trigger mechanism and needed almost 6 minutes for the full release of its content (figure 9), but this response did not mean an early trigger if compared with the bigger ratio ones (0.036 and 0.057) because, as we can see in figure 9, the first two responses outcome from them, regarding their subtle release throughout time. Therefore, from this test we could not relate an early trigger with an easy content release but yet we did observe effectively more time (10 minutes) to disintegrate the bigger ratio ones.

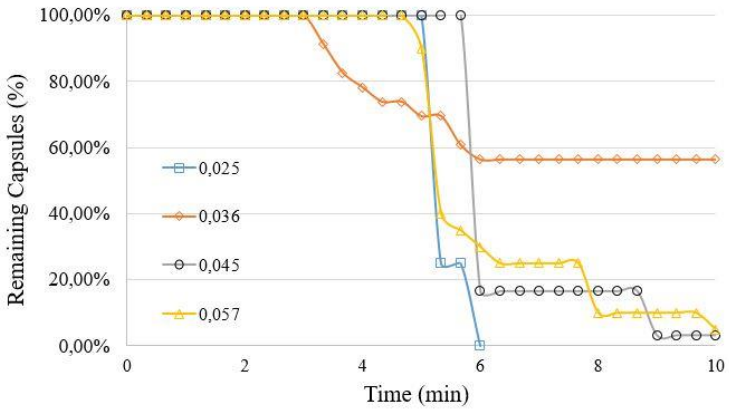

Figure 9. Remaining capsules test from group 1 with an average full release time of 9 minutes.

The second group (figure 10) shows a similar trigger event. We can an observe an abrupt drop in the percentage of the remaining capsules for the smaller t-D ratio ones (0.022), as with group 1, but an increase in full release time of 1.34 minutes. As for the bigger ratio ones, the release is rougher if we compare with the first group but yet more subtle than the smallest in this group.

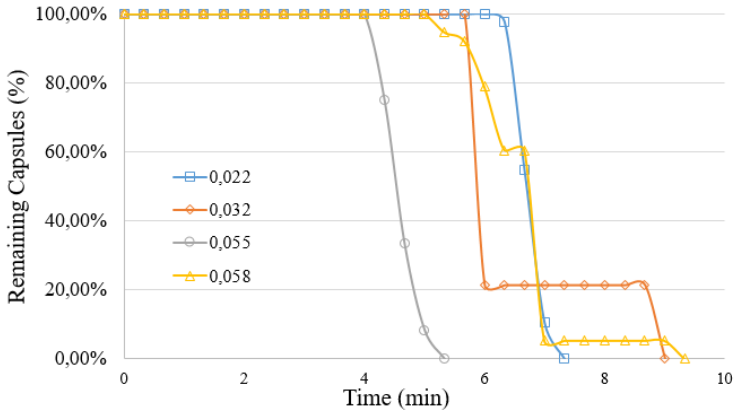

Figure 10. Remaining capsules test from group 2 with an average full release time of 7.6 minutes

From this data, we can observe a drop in the average residence time needed to fully release the gellan-based microcapsule content. While group 1 completed a full release within 9 minutes, group 2 decreased this average time to 7.6 minutes. Probably this tiny difference between time could be explained by the close t-D ratios used in this experiment, when a bigger ratio (t/D>0.1) and gaps between them would have suited better for this experiment. It is good to mention that some samples escape from the average behavior like samples #2, with an unobserved full inner content release, and #7 having the same properties of #8 but did not repeat a similar release time. All these potential faults could be explained by some impurities in the sample or volume differences poured in the device between tests or even imprecise judgment whether happened the trigger activation or not.

4. CONCLUSION

The purpose of the current study was to determine controlled conditions at which gellan gum-based microcapsules are destroyed by a temperature trigger. The results of this investigation show that the inner content release is a function of the environment temperature and capsule size.

In this work we analyzed the low acyl (LA) gellan form capsules, divided in two groups of diameters with different thickness-diameter ratios (t/D), and the partial results show that these ratios can be a promising line of study if we compare them with bigger ratio differences between them to reinforce the temperature trigger event as a controlled release way to deliver the inner content. Furthermore, we have reached an optimum temperature of 75 ºC where almost in all trigger tests we could successfully disintegrate the gellan-based shell. Lower temperatures events seemed to activate the trigger in a random and slower behavior at which in none of the tests all capsules degraded.

As for future works, another way of comparison is to study the elastic and soft natural high acyl (HA) form of the gellan gum adding a fluorescent dye in the inner phase of the capsules and increase the acidic conditions of the medium that particles are inserted.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

Abbaspourrad, A., Carroll, N.J., Kim, S.H., Weitz, D.A., 2013. “Polymer microcapsules with programmable active release”. J. Am. Chem. Soc. 135,7744-7750.

Amstad, E., 2017. “Capsules: their past and opportunities for their future”. ACS Macro Lett. 6, 841–847

Bacelar, A.H., Silva-Correia, J., Oliveira, J.M., Reis, R.L., 2016. “Recent progress in gellan gum hydrogels provided by functionalization strategies”. J. Mater. Chem. B. 4, 6164–6174.

Chen, P.W., Erb, R.M., Studart, A.R., 2012. “Designer polymer-based microcapsules made using microfluidics”. Langmuir 28, 144–152.

Costa, A.L.R., Gomes, A., Ushikubo, F.Y., Cunha, R.L., 2017. “Gellan microgels produced in planar microfluidic devices”. J. Food Eng. 209, 18–25

Datta, S.S., Abbaspourrad, A., Amstad, E., Fan, J., Kim, S.H., Romanowsky, M., Shum, H.C., Sun, B., Utada, A.S., Windbergs, M., Zhou, S., Weitz, D.A., 2014. "25th Anniversary Article: Double emulsion templated solid microcapsules: mechanics and controlled release". Adv. Mater. 26, 2205–2218.

Fisher, S., Lee, H., Kallos, M.S, Hunter, C.J., 2010. "Degradation and mechanical properties of gellan gun for musculoskeletal tissue engineering". University of Calgary. Poster N1908. ORS2011 Annual meeting.

Graham, S., Marina, P.F., Blencowe, A., 2019. "Thermoresponsive polysaccharides and their thermoreversible physical hydrogel networks". Carbohydr. Polym. 207, 143–149.

Michelon, M., Leopércio, B.C., Carvalho, M.S. 2020. "Microfluidic production of aqueous suspensions of gellan-based microcapsules containing hydrophobic compounds". Chem. Eng. Science. 211. 115314

Michelon, M., Huang, Y., de la Torre, L.G., Weitz, D.A., Cunha, R.L., 2019. "Single-step microfluidic production of W/O/W double emulsions as templates for bcarotene-loaded giant liposomes formation". Chem. Eng. J. 366, 27–32.

Mao, R., Tang, J., Swanson, B.G., 2000. "Texture properties of high and low acyl mixed gellan gels". Carbohydrate Polymers. Vol.41, Issue 4, 331-338

do Nascimento, D.F., Avendaño, J.A., Mehl, A., Moura, M.J.B., Carvalho, M.S., Duncanson, W.J., 2017. "Flow of tunable elastic microcapsules through constrictions". Sci. Rep., 7:11898.

Padsalgikar, A. D., "Plastic in Medical Devices for Cardiovascular Applications". Plastic Design Library, 83-102.

Sun, B.J., Shum, H.C., Holtze, C., Weitz, D.A., 2010. "Microfluidic melt emulsification for encapsulation and release of actives". App. Mat. Int. Vol 12. 3411-3416.

Werner, J.G., Nawar, S., Solovev, A.A., Weitz, D.A., 2018. Hydrogel microcapsules with dynamic pH-responsive properties from methacrylic anhydride. Macromol. 51, 5798–5805.

Yamamoto, F., Cunha, R.L., 2007. "Acid gelation of gellan: effect of final pH and heat treatment conditions". CarbHydro.Polym. 68, 517–527.

Zhao, Y., Shum, H.C., Adams, L.L.A., Sun, B., Holtze, C., Gu, Z., Weitz, D.A., 2011. "Enhanced encapsulation of actives in self-sealing microcapsules by precipitation in capsule shells". Langmuir 27, 13988–13991.

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