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Effect of adding chitosan capsules produced by ionotropic gelation on the mechanical properties of mortars

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ABSTRACT

To ensure the viability of bacteria in the biomineralization process for crack filling and healing, protection from the heat (generated by cement hydration) and the retraction (during hardening) of the microorganisms is required. Superabsorbent polymers, as chitosan, known for their resistance during mixing and water storage capacity, are suitable bacteria carriers. This study aimed to determine the optimal percentage of chitosan capsules and its effects on the physical and mechanical properties in mortars. Mortar formulations containing 0.5%, 1.0%, and 1.5% of chitosan capsules (relative to the total cement weight) were developed keeping the original granulometric distribution. Analysis of variance (ANOVA) for each individual property and *post-hoc* tests with corrections of Bonferroni and Tukey demonstrated that the formulation significantly impacted both properties through aging. Formulations AQ-0.5C and AQ-1.0C (0.5% and 1.0% of capsules respectively) maintained the flow levels comparable to the original formula. AQ-0.5C showcased the most favorable outcomes, exhibiting mechanical resistance up to 10% higher than the capsule-free original formula. Even slightly inferior results, AQ-1.0C still presented a higher strength mechanical up to 5% over the original formula. These results indicate the potential use of the ionotropic gelation to produce spores with bacteria to be applied in biomineralization for sealing cracks.

Keywords: Mortar; cracks; bacterium; biomineralization; chitosan.

1. INTRODUCTION

Mortars are composites used for construction formed by the combination of aggregates, Portland cement, and water [1]. It is a material with good compression resistance; however, its low tensile strength allows the appearance of cracks. The deterioration through time occurs due to several mechanisms, such as thermal tension, expansive chemical reactions, carbonation corrosion, and freeze-thaws cycles [2, 3]. The cracks reduce durability of mortars since they allow penetration of water and harmful chemical agents [4].

Active treatments are self-healing techniques that can seal cracks immediately after their formation, regardless of their position or orientation [2, 5]. In order to implement the active treatment using bacteria, calcium carbonate is produced by biomineralization, that is, the formation of minerals by living organisms, usually due to their microbial activity [6].

In self-healing based in capsules, the capsules are responsible for protecting the bacteria mechanically and releasing them after being activated by the cracks, moisture, air, or pH change in matrix [7]. The capsules must be biocompatible, not affect the mechanical properties of the material, be strong enough to support the mixing process and release the healing agent upon cracks; moreover, the capsules must be evenly distributed in the material, so that cracks have a better chance of hitting the capsules, regardless of their position [8].

Several materials were studied as spore capsules, such as diatomaceous earth, silica gel, polyurethane, melamine microcapsules, graphite nanoplatelets, light aggregates and hydrogel capsules. The advantage of

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using hydrogel as a carrier for spores is that it works as a water reservoir, providing water in dry periods to promote metabolic activity [8].

Chitosan spheres have been studied in pharmaceutical industry, aiming the release of drugs such as amoxicillin and tetracycline [9–11]. Ionotropic gelation used to prepare the chitosan spheres is a simple process, consisting of cross linking by electrostatic interaction. In this process, chitosan is diluted in acid solution; this solution is then dripped into basic solution, as sodium tripolyphosphate (TPP). TPP reacts electrostatically with chitosan, causing ionotropic gelation and precipitation of chitosan as spheres [12].

When considering the addition of capsules to mortar formulations, one must pay attention to possible negative effects to rheological, physical and mechanical properties of the material. The adequate formulation, resulting in efficient packing of the particles, has a direct effect in several of its properties. Obtaining dense packing is related to the continuous filling of empty spaces between particles by other particles with smaller diameter. Attention must also be paid to the introduction of particles greater than the ones present, as it may lead to the appearance of new empty spaces, leading to an increase in porosity. Thus, not only the size of the particles, but also their distribution must be controlled, in order to determine the increase or not of packing density [13].

Several papers evaluated the addition of bacterial to mortar aiming to determine their influence on the properties of the product using different types of capsules to protect the spores [3, 14–18, 19–25]. However, in these papers, the addition of the capsules is made solely on top of standard formula, disregarding its effect on rheological and mechanical properties of the mortar. Simple addition of capsules may cause segregation, depending on the granulometric distribution of the added capsules, which may result in increased porosity and decreased flow, density, and mechanical resistance.

In order to avoid this problem, it is possible to perform granulometric compensation, that is, when adding the chitosan capsules, an equivalent amount of aggregate with similar size is removed. When compensation is performed, the maintenance of grain size distribution of the original formula can be guaranteed, minimizing the effects of capsule addition in the properties of mortars. It is possible to effectively measure such effects by performing multivariate analysis of variance (MANOVA) in which individual results of physical and mechanical properties, for each formulation, are analyzed and, as a result, it is obtained an indication of one or more formulas with statistical results different from others. The type of influence, positive or negative, may then be accessed by complementary statistical tests, such as analysis of variance (ANOVA) individual for each property, and post hoc tests with Bonferroni and Tukey corrections [26], for instance.

The objective of this paper was to evaluate the efficiency of the addition of chitosan capsules prepared by ionotropic gelation, as potential carriers for bacteria in mortar formulations, as well as its effect on the mechanical property. Nevertheless, the capsules were not simply added to the formulation, but a granulometric compensation was also realized. Multivariate analysis of variance and further statistical tests were performed to evaluate the influence on the properties of fresh and hardened material, aiming the maintenance of properties during application and possible liberation of bacteria when a crack occurred.

2. MATERIALS AND METHODS

Figure 1 illustrates the procedures adopted for the development of this paper, to select – by evaluation of mechanical, physical and rheological properties – the compositions with chitosan capsules that presented the best set of results, with the least effect on the properties of original mortar.

2.1. Raw material analysis

To prepare all mortar formulations, it was used Portland cement CP-II E 25, from Cauê, sand in different grain sizes namely Coarse, Regular-Coarse, Regular-Fine and Fine, from IPT, according to the limits stablished by standard ABNT NBR 7214:2015 [27], and chitosan spheres.

The four sizes of sand and the capsules were analyzed using sieves Tyler #7, 9, 10, 16, 32, 100, 200 and lower than 200, to evaluate grain size distribution and determine which size of sand is closer to the size of the capsules produced.

2.2. Synthesis of chitosan capsules

In order to prepare chitosan capsules, it was used 85%-purity deacetylation chitosan from Polymar Ciência e Nutrição, and sodium tripolyphosphate tech grade from Indústria Química Anastácio, with minimum purity of 94%. Raw materials were chosen considering cost-benefit in order to reduce the cost of production of the capsules.

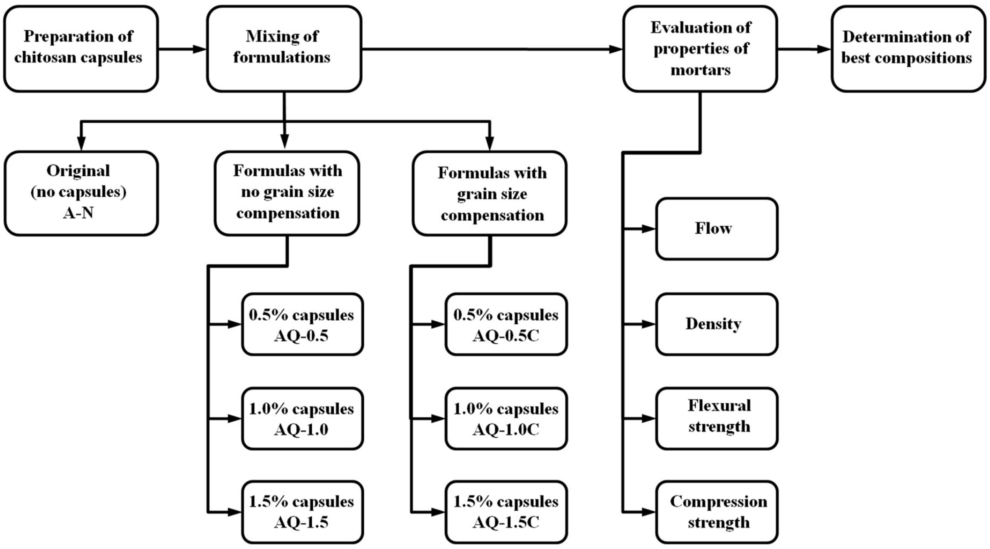

Figure 1: Methodology detailing.

Table 1: Formulations (in %) of mortar with chitosan capsules.

COMPONENTS	STANDARD	WITHOUT COMPENSATION			WITH COMPENSATION		
	A-N	AQ-0,5	AQ-1,0	AQ-1,5	AQ-0,5C	AQ-1,0C	AQ-1,5C
Portland Cement	22,05	22,05	22,05	22,05	22,05	22,05	22,05
Water	11,81	11,81	11,81	11,81	11,81	11,81	11,81
Sand							
Coarse	16,54	16,54	16,54	16,54	16,43	16,32	16,21
Regular-Coarse	16,54	16,54	16,54	16,54	16,54	16,54	16,54
Regular-Fine	16,54	16,54	16,54	16,54	16,54	16,54	16,54
Fine	16,54	16,54	16,54	16,54	16,54	16,54	16,54
Capsules	-	0,11	0,22	0,33	0,11	0,22	0,33

Chitosan solution (QS) 2% (m/v) was prepared by adding the polymer into acetic acid solution 1% (v/v), under mechanical agitation until complete dissolution. This solution was then dripped into a sodium tripolyphosphate solution (TPP) 2% (m/v) to form the spheres. After the precipitation, the spheres were dried at 110°C during 3 hours for posterior incorporation in mortar formulations.

2.3. Formulations with chitosan capsules

It was prepared mortar formulations with 0.5%, 1% and 1.5% chitosan capsules in relation to cement weight. For the formulas without granulometric compensation, the number of capsules was simply added on top of the original formula. For the formulas with granulometric compensation, it was removed part of coarse sand equivalent to the number of capsules added, as its grain size is closest to the capsules. The formulas with chitosan (AQ) are listed in Table 1, in comparison to standard formulation (A-N). The percentage of capsules added is indicated for each case, and the formulas with granulometric compensation are indicated with a "C" at the end.

2.4. Evaluation of mortar properties

The tests for evaluation of mortar properties were performed according to standards ABNT NBR 7215:2019 [28] and NBR 16738:2019 [29].

For each formula, it was evaluated rheological properties by the flow measurement. The mortar was placed in the flow table within an aluminum cone with superior diameter of 70 mm and inferior diameter of 100 mm. After filling the cone and compacting the mortar, the cone was removed, and it was applied 25 taps in 15 seconds; the spread was measured using a pachymeter.

In sequence, 9 specimens with dimensions 160 × 40 × 40 mm were moulded, which were evaluated in three different ages (7, 28 and 91 days). After demoulding, the specimens were kept immersed in water until it was time to determine the physical and mechanical properties.

To determine the formulation with the best set of results, statistical analysis was performed in the measurements of density (DA), flexural strength (RFTA) and compression strength (RCTA) for each age (7, 28 and 91 days). It was performed multivariate analysis of variance (MANOVA), with $\alpha = 0.05$. The tests were followed by analysis of variance (ANOVA) for each individual property, and *post-hoc* tests with corrections of Bonferroni and Tukey [26].

Microscopic-optical images were obtained, using an optical microscope from Quimis, highlighting the distribution of the capsules in the mortar sample and showing the effect of the biomineralization with the formation of calcium carbonate in the cracks.

3. RESULTS AND DISCUSSION

3.1. Raw material characterization

Figure 2 shows chitosan capsules obtained after drying for 3 hours at 110°C. The ruler was used as a reference to show the size of the capsules.

Grain size distribution of the different sizes of sand and the chitosan capsules are listed in Table 2. It is noticeable that the distributions of the coarse sand and the capsules were similar. Thus, to perform granulometric compensation in mortar formulas the capsules were added, and an equivalent amount of coarse sand was

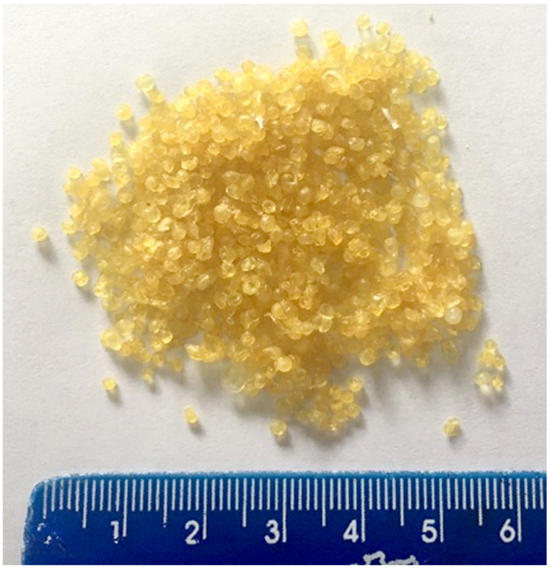

Figure 2: Dried chitosan capsules sizes.

Table 2: Accumulated grain size analysis of the different sizes of sand and chitosan capsules.

SIEVE (TYLER)	OPENING (mm)	CAPSULES	SAND			
			COARSE	REGULAR COARSE	REGULAR FINE	FINE
7	2,80	3,03	0,00	0,00	0,00	0,00
9	2,00	21,21	15,40	0,00	0,00	0,00
10	1,70	40,40	41,36	0,00	0,00	0,00
16	1,00	100,00	99,73	34,52	0,00	0,00
32	0,50	100,00	99,84	99,59	25,38	0,00
100	0,15	100,00	99,84	99,59	99,75	96,66
200	0,075	100,00	99,89	99,73	99,75	100,00
Total	–	100,00	100,00	100,00	100,00	100,00

Table 3: Flow found for the formulas tested.

FORMULA	FLOW (mm)
A-N	193,9 ± 0,7
AQ-0,5	188,3 ± 0,6
AQ-0,5C	193,1 ± 0,6
AQ-1,0	179,2 ± 1,2
AQ-1,0C	196,9 ± 0,9
AQ-1,5	182,3 ± 1,3
AQ-1,5C	186,1 ± 0,7

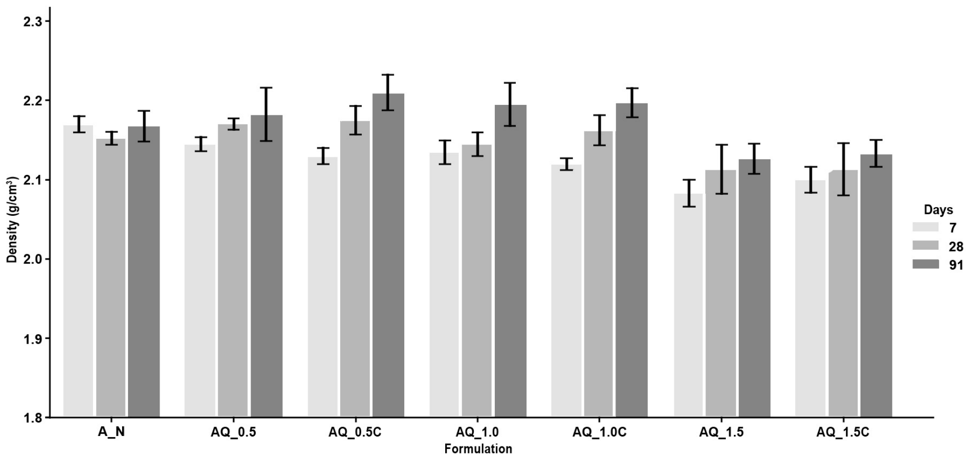

Figure 3: Density of formulas after 7, 28 and 91 days.

removed. Compensation was extremely important because, by replacing part of coarse sand with capsules, the grain size distribution could be maintained close to the original formula, diminishing any negative influence that could be generated by the addition of the capsules in the composition.

3.2. Evaluation of mortars containing chitosan spheres

3.2.1. Rheological properties

Table 3 shows the results found for flow of each formula. It can be noticed that, in a general context, formulas with granulometric compensation presented flow values higher than those without compensation. Still, formulas AQ-0.5C and AQ-1.0C had values quite close to the ones found for the standard formula, which confirms that the compensation is necessary to maintain the original properties of the mortar. From the 1.5% capsule addition, however, it can be observed that the flow was affected, which could indicate the beginning of segregation.

3.2.2. Density

Figure 3 shows the development of density for each formulation in each age evaluated (7, 28 and 91 days). As expected, with the increase of capsules present in the formula, there was a decrease in the value of density, due to the difference between the density of the mortar and the capsule.

Regarding time, it can be noticed that for each formulation containing chitosan capsules there was an increase of density of each age, whereas for the standard formula, the values tended to remain close in all three ages evaluated. From this observation, it is possible to conclude that the addition of capsules tends to affect density in early ages – while the hydration reactions are still being established; however, this influence tends to decrease over time.

Finally, it is possible to observe that formulations with similar capsule levels also tended to present similar behavior; however, the granulometric compensation resulted in specimens with lower variability in the results. After 91 days, formulations containing 0.5% and 1.0% capsules presented density results equal or superior to the ones obtained for the standard formula.

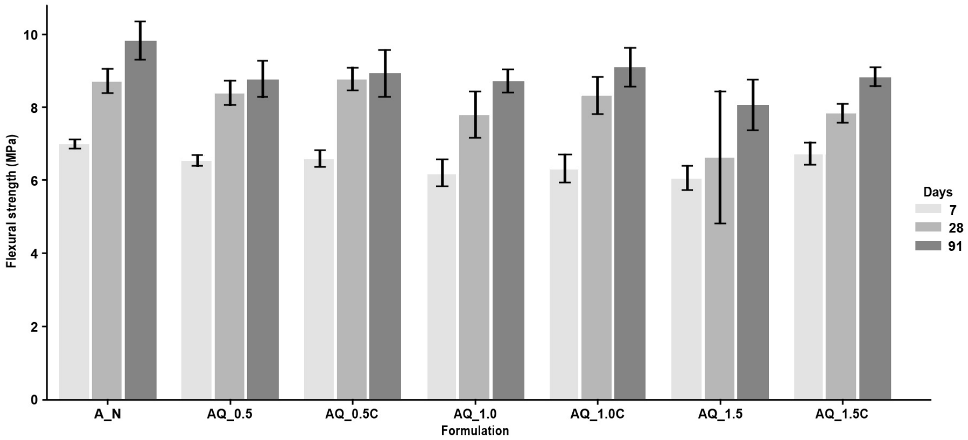

Figure 4: Flexural strength of formulations after 7, 28 and 91 days.

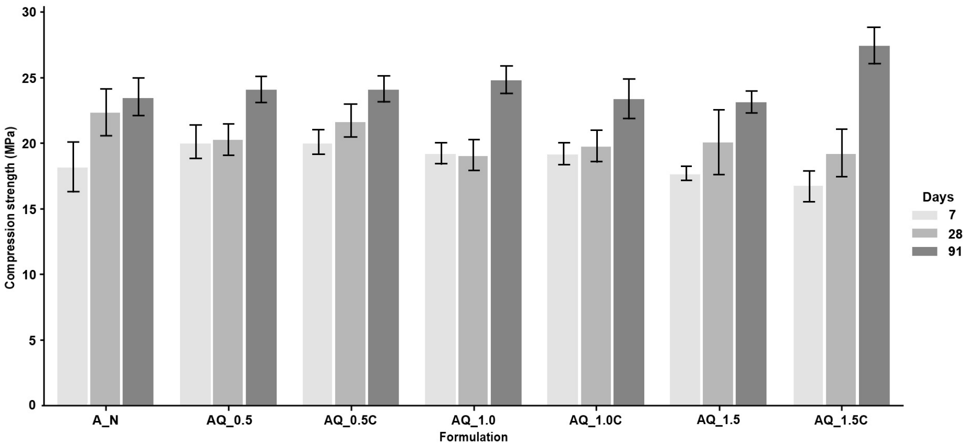

Figure 5: Compression strength of formulations after 7, 28 and 91 days.

3.2.3. Flexural strength

Figure 4 shows the development of flexural strength for each formulation, at each age evaluated (7, 28 and 91 days).

For flexural strength property, it can be noticed that the value of resistance obtained for the formulations with capsules were slightly inferior to those observed in the standard formulation. However, by observing the behavior over time, formulations with capsules presented a leap in the resistance value after 28 days, which tends to stabilize after 91 days. The standard formulation presented a more gradual development over the time, leading to the conclusion that the capsule addition has a greater effect in early ages, being less significant when the hydration reactions are completed.

Besides that, it is also possible to observe the effect of granulometric compensation in the variability of the results. Even after 28 days, when the reactions are complete, the formulation AQ-1.5 still presents great dispersion in the values of flexural strength as a result of the segregation observed during flow evaluation. Nonetheless, granulometric compensation helped to control this segregation in formulation AQ-1.5C.

As well as in density, formulations with 0.5% and 1% capsules presented flexural strength values closer to the ones observed in the standard formulation.

3.2.4. Compression strength

Figure 5 shows the development of compression strength for each formulation at each age (7, 28 and 91 days).

The addition of capsules provoked the same effect in compression strength as it was observed for flexural strength in early ages. However, in this case, resistance values were also lower after 28 days, and only after 91 days, values closer to the standard formulation were obtained. Higher amounts of capsules added resulted in

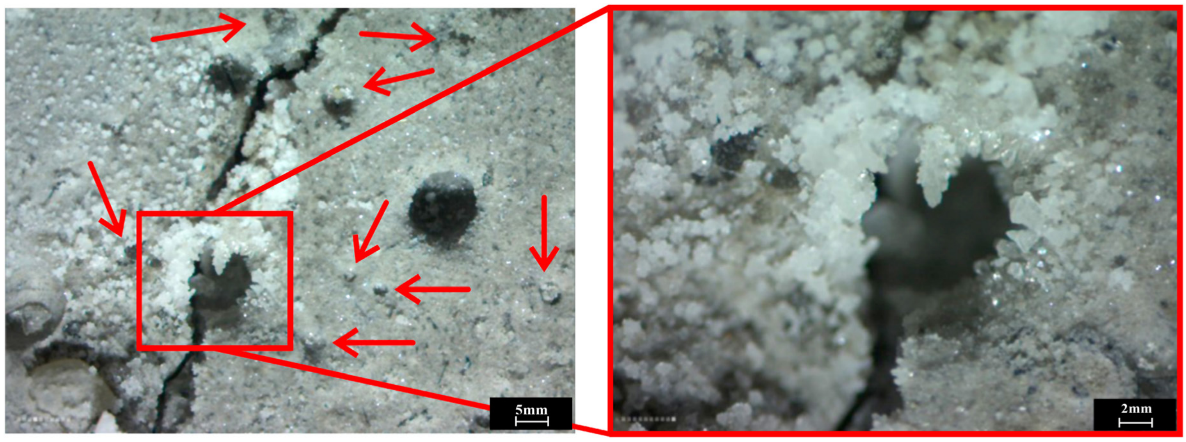

Figure 6: Distribution of capsules in a sample and the deposition of $CaCO_2$. Image in the left with zoom of 65x and image in the right with a zoom of 160x.

lower values of compression resistance after 7 and 28 days, and a higher increase in resistance after 91 days. In short, while the hydration reactions are still taking place, the higher the number of capsules present, the greater is its influence in compression resistance.

Standard formulation presented a more gradual development of resistance over time and, unlike the formulations with capsules, the value of compression strength tends to stabilize after 28 days.

The behavior of specimens when subjected to compression was similar to flexural strength regarding the variability of the results of formulations without compensation. Formulation AQ-1.5 still presented greater dispersion in the values of compression strength when compared to the other formulations; however, for this property the variability was lower, since the mortar is a material more resistant to compression than flexion, and the influence of segregation is slightly lower. Yet, this compensation resulted in more consistent specimens.

For this property, all formulations presented compression strength values superior to the ones observed for the standard formulation, however it was necessary more than 28 days to observe this behavior.

3.2.5. Optical microscopy analysis (OM)

Optical microscopy was used to verify the distribution of the capsules in the samples and to evidence the deposition of calcium carbonate by the bacteria in the cracks. The result of the optical analysis can be seen in Figure 6.The capsules are discriminated by the arrows. An increase in the zoom in the crack area was done to show the deposition of $CaCO_3$.

3.2.6. Statistical analysis

In literature there are several papers where it is performed the mechanical evaluation of mortar specimens after the addition of capsules containing bacteria [22, 23, 30]. However, the mixing and evaluation procedures are different from the one stablished by standard ABNT NBR 16738 [29] used as a reference in this study. Thus, the results differ greatly from the ones obtained: compression strength results for the mortar prepared according to the ABNT standard are approximately 22 MPa after 28 days, while the results found in literature vary from 40-70 MPa, which are closest to what is found for concrete.

Since the standard ABNT NBR 16738 [29] does not specify minimum values for flexural or compression strength, all statistical evaluations were performed considering the standard formulation (reference) as stablished in it. The formulation was considered as adequate when the results were statistically equal or superior to those obtained from the standard formulation.

For all properties, it can be observed that the addition of 1.5% capsules, with or without compensation, led to greater variability in the results, possibly due to segregation, as indicated by flow evaluation.

Results obtained from multivariate analysis of variance (MANOVA) in all three properties after 7 days using Pillai's trace indicated significative effect of formulation ($V = 2{,}32$, $F(18{,}42) = 8$, $p < 0{,}05$). The formulations which presented the best set of results after 7 days were AQ-0.5 and AQ-0.5C.

After 28 days, MANOVA using Pillai's trace also indicated significative effect of formulation in the set of results ($V = 1{,}55$, $F(18, 42) = 3$, $p < 0{,}05$). In this age, the hydration reactions of cement were complete, and the behavior and influence was already clear. Formulations AQ-1.5 and AQ-1.5C presented performance inferior to the others after 28 days. Formulations with 0.5% and 1% of capsule addition were equivalent to the standard one, especially if granulometric compensation was performed, ensuring that the capsule influence was as low as possible.

After 91 days, all hydration reactions were fully consolidated, and it can be observed that formulations with addition of 0.5% and 1% of capsules presented results greatly similar to standard formulation in all properties evaluated. The results obtained from MANOVA using Pillai's trace indicated significative effect of formulation ($V = 2{,}28$, $F(18, 42) = 7$, $p < 0{,}05$). At this age, only formulation AQ-1.5 presented results inferior to others.

4. CONCLUSION

By analyzing the set of results, in all ages evaluated, it can be observed that the formulations with chitosan capsules that presented the best results were AQ-0.5, AQ-0,5C and AQ-1.0C. It is clear, according to the tests performed, that the compensation enhances the results for each property. Given two formulations with the same level of capsule addition, compensation increased up to 7% the mechanical resistance after 28 days. When compared to standard formulation (reference), gains up to 10% in compression resistance after 28 days could be obtained.

As for the rheology of the material, formulations AQ-0,5C and AQL-1,0C presented results statistically equal to those observed for the standard formulation. Besides, when compared to formulations with the same level of capsule addition, but without granulometric compensation, flow gain was approximately 2.5% to 10%.

Hence, according to the results obtained and the detailed statistical analysis, it is recommended the use of formulation AQ-0.5C, which was the formula that presented the best set of results among the tests performed. Formulation AQ-1.0C is also indicated, since it presented good results and led to higher concentration of capsules, which can be beneficial to enable self-healing.

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