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Rheological and Morphological Properties of Carboxymethylcellulose/Starch Blends with or without ZnO and Their Applications as Inoculant Carrier

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Summary: Using controlled shear-rate testing ramps, this study investigates the rheological properties of carboxymethycellulose (CMC)/starch blends, with and without zinc oxide (ZnO). Viscosity decreased as the amount of starch increased and after adding 1% ZnO. The creep and recovery tests indicated that, with increasing starch content, there was a decrease in elasticity and the viscosity zero shear rate. The results also showed, in the temperature range between 25 and 70 °C, an inversion occurred in the elastic and viscous moduli behavior, obeying the Arrhenius equation. The addition of ZnO affects the viscoelastic behavior and the morphology of the blends. The best survival results were obtained for samples 50/50 and 60/40 wt% (CMC/starch).

Keywords: biocompatibility; blends; miscibility; polysaccharides; rheology

Introduction

Recently industries have shown a demand for environmentally friendly polymers that have good rheological and mechanical properties. This demand has motivated researchers to search for biodegradable polymers with non-toxic and recyclable characteristics. Such polymers are being sought from renewable resources and at low cost. In this context, researchers have shown an interest in the properties exhibited by carboxylmethylcelullose (CMC) and their blends.[1–5] The electron density, present in the backbone chain, may change the macroscopic properties of the solution, making them useful as rheology modifiers. This backbone chain contains hydroxyl groups, which can interact strongly with other polar groups and water.[6,7] Because of its molecular structure, CMC may be blended with starch to obtain gels with improved physical and mechanical properties.[8] CMC has been used in water treatment as a thickening agent, as a way to stabilize the clay suspensions, as support for ion-exchanges in membranes, lotions, and paints, and as a protective colloid.[9,10]

Although several works reported the use of CMC and their blends for many technological applications, the information's about CMC/starch blends are still scarcely found, in particular for agriculture applications. The starch is an important biopolymer and it has also extensively applications technological. Researchers have extensively studied starches mixed with synthetic polymers.[11–12] The goal of mixing starches with other polymers is to improve the starch's physical and mechanical properties. This objective is often unachieved due to incompatibility between polymers, resulting a decrease of the physical-mechanical properties of blend. In such cases, we can improve the

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compatibility, resulting from increasing the phase's interaction, by adding interfacial agents. Compatibility between the polymers is critical to the blends' physical-mechanical performance. Good compatibility increases the interaction between the phases, helping the material retain its physical properties.[13]

The goal of the present paper is investigate how the addition of zinc oxide (ZnO) affects the rheological properties of CMC/starch blends. We will pay special attention to the morphology and bacteria cell survival to determine the blends' usefulness as an inoculant carrier for agriculture applications.

The present study compares the rheological behavior of CMC/starch blends in a temperature range of 25 to 70 °C. It also evaluates how the zinc oxide (ZnO) influences flow activation energy and viscoelastic properties.

Experimental Part

Materials

Carboxymethylcellulose has a substitution range of 0.65–0.90. Our stock of it was supplied by Quimesp. The starch and zinc oxide were supplied by Vetec S.A.

Sample Preparation

The carboxymethylcellulose-starch (CMC/starch) blends were prepared using stirred mixers 600 rpm for 10 minutes at 25 °C, Aqueous solutions were prepared from 100/0 up to 20/80 wt% (CMC/Starch) with and without 1.0 wt % of ZnO. The concentration of polymer was 64 grams/liter. The samples were sterilized for 30 minutes at a constant temperature and pressure of 100 °C and 13 KPa.

Rheological Measurements

The rheological measurements of the CMC/starch blends, with and without ZnO (1%wt), were carried out using an oscillatory rheometer model RheoStress 1 (Haake-Germany), having a low inertia parallel plate geometry PP35Ti with a gap of 1.00 mm. The samples were investigated by controlled shear rate testing ramps from 0 up to 1000 1/s. To obtain the viscosity profile as a function of the shear rate, the experimental data were fitted by an Ostwald de Waele rheological model, defined by Equation (1).[14] The temperature was controlled by a DC10 K10 water bath model (Haake-Germany) connected to the bottom plate. All experiments were conducted at a temperature range of 25 to 70 °C.[14]

$$\tau = \mathrm{k}.\dot{\gamma}^{\mathrm{n}} \qquad (1)$$

Where τ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (1/s), *k* is the consistency index (Pa.s) and ^{n} is the flow behavior index (dimensionless).

Apparent Viscosity Determination and Effect of Temperature

We determined the apparent viscosity ($\eta_{a,100}$), at a shear rate of 100 1/s, using linear interpolation. Linear interpolation utilizes a method of curve fitting using linear polynomials, as shown by Equation (2). This is done, using Rheowin 3 software, on a viscosity curve as a function of the shear rate.

$$\mathrm{y} = \mathrm{a} + \mathrm{bx} \qquad (2)$$

Where *y* is the dynamic viscosity (Pa.s), *x* is the shear rate (1/s), *a* is the linear coefficient (Pa.s) and *b* is the angular coefficient (Pa.s^2).

We also studied how temperature (25–70 °C) influenced apparent viscosity values. At a specific shear rate value, this influence may be described by the Arrhenius relationship, Equation (3),[15] where the apparent viscosity decreases exponentially with temperature.

$$\eta_{a,100} = \mathrm{A}.\exp(\mathrm{E_a}/\mathrm{RT}) \qquad (3)$$

Where $\eta_{a,100}$ is the apparent viscosity (Pa.s) at 100 1/s, *A* is a constant (Pa.s), *R* the gas constant (8,3144 J/mol.K), *T* the absolute temperature (*K*) and E_a the activation energy (J/mol).

Frequency Sweep Test

The stress amplitude for all the dynamic tests, and for all samples according to previously carried-out amplitude sweep tests (not shown), was selected within the linear viscoelastic region (LVR) as 5 Pa. Before testing, each sample was transferred to a rheometer plate and left for two minutes so as to release residual stress.[16] To obtain the complex viscosity profile as a function of the angular frequency, we conducted the dynamic tests using a frequency sweep mode under a stress amplitude of 5 Pa at 25 °C, over an angular frequency range of 0.06 to 135.40 rad/s.

Creep and Recovery Test

Generally, in a creep and recovery test, one can replicate elastic deformation with a spring model and viscous flow with a dashpot model. We can represent different kinds of viscoelastic materials through the quantity of springs and dashpots and by how we arrange their connections in the sample body.[17] Creep and recovery tests were carried out under a shear stress of 5 Pa at 25 °C. How the strain varied in response to the applied stress was measured for a period of three minutes. The stress was then removed and, to observe structure recovery, changes in strain were measured for another three minutes. Using the evaluation tool from Rheowin 3, it was also possible to provide viscosity zero shear rate and relaxation time determination.

Scanning Electron Microscopy

The morphology of the CMC/starch blends was characterized with the help of SEM, using a JEOL JSM 6490 - LV Scanning Electron Microscope. The samples were sputter-coated with gold.

Survival Test

The rhizobia strain BR 3267 of *Bradyrhizobium japonicum* was obtained from the diazotrophic culture collection of Embrapa Agrobiologia.[18] The survival tests were carried out according to a methodology described in the literature.[19]

Results and Discussion

Flow Behavior of CMC-ZnO/starch Blends

The shear stress (τ) versus shear rate ($\dot{\gamma}$) data for both compatibilized and non-compatibilized blends, at 25 °C, fit well the Ostwald de Waele rheological model [Eq. (1)], a model used extensively to describe the flow properties of non-Newtonian fluids.[20] As shown in Table 1 and 2, all blends exhibited shear thinning behavior. The flow behavior indexes (n) showed values as low as 0.32–0.10. The strong influence of starch concentration was exhibited in the fact that the magnitudes of flow behavior indexes (n) increased with starch content. It is well known that at very low concentrations, polymers such as CMC are in their most extended conformation; molecules are widely separated and the viscosity shows slight shear rate dependence. At higher concentrations, on the other hand, the extended chains start to overlap and viscosity increases. Then, the coiled chains start to overlap and entangle, the physical contact actually changing the flow behavior. In this study, we have used a highly concentrated (64 grams/liter)

Table 1.
Apparent viscosity dependence of non-compatibilized CMC/starch blends describers by Oswald de Waele model and apparent viscosity (η_{100}; T = 25 °C).

CMC Content (%)	Apparent Viscosity(Pa.s)	^{a} K (Pa.s)	^{b} n	^{c} R^2
100	10.57	618.90	0.10	0.90
80	8.14	452.00	0.14	0.93
60	3.22	112.40	0.22	0.99
50	2.62	88.22	0.23	0.99
40	1.33	30.86	0.32	0.99

Table 2.
Apparent viscosity dependence of compatibilized CMC/starch blends describers by Oswald de Waele model and apparent viscosity (η_{100}; T = 25 °C).

CMC Content (%)	Apparent Viscosity(Pa.s)	[a] K (Pa.s)	[b] n	[c] R^2
100	9.24	534.40	0.10	0.90
80	6.12	301.50	0.14	0.96
60	3.10	108.20	0.22	0.98
50	1.80	51.76	0.27	0.99
40	1.31	33.16	0.29	0.99

polymer solution. The results displayed in Table 1 are in good agreement with theoretical predictions. The viscosity values show strong shear rate dependence.[10,21]

The rheological behavior of the 100% CMC composition closely resembles that of polyelectrolytes. It is well known that CMC is a water-soluble polymer and, depending on its concentration in aqueous solution, its structure may exhibit a high propensity for gel formation. This occurs due to the strong intermolecular interactions provided by hydrogen bonds established among the water, carboxyl, and hydroxyl groups found on a CMC macromolecule. Starch, on the other hand, is a neutral hydrocolloid. The formation of a stable structure is possible only through the polymeric chains' attractive interaction.[22–23]

Comparing the viscosity values of the CMC/starch blends with and without ZnO, it can be observed that the addition of 1% ZnO reduce the apparent viscosity ($\eta_{a,100}$) Table 2. It indicates the structure entanglement is decreased. As shear rate increases, the organized structures as CMC 100/0 are lead to a destructuration process, decreasing viscosity.

When the amount of starch in the blend increase, the macromolecular structure of the blends become less entanglement and the viscosity decrease. The increase of starch content on the blend reduces the dependence of viscosity with shear rate and the rheological characteristic approximates to Newtonian behavior. The starch is a biopolymer neutral and in absence ions only hydrophobic interactions are present. The addition of Zn^{++} ions to the systems will decrease gelation by decreasing the hydrophilic interactions between starch macromolecules, reducing the viscosity of the solution.[24]

Effect of Temperature on Apparent Viscosity

Table 3 shows the activation energy of CMC-starch blends. It can be observed that, for compositions rich in CMC especially, the addition of ZnO reduces the activation energy. This can be attributed to the interactions between zinc ions and carboxyl groups of CMC macromolecules. The carboxyl-ZnO reaction improves the interaction between carboxyl-hydroxyl (starch), improving its interaction with the starch phase. The increased starch content reduced the carboxyl-ZnO interaction probability. If the ZnO-CMC interaction is reduced, less organized CMC structure was present in the mixtures, than increase of the activation energy can be expected. It was observed that the activation energy increased at 50/50 wt%, followed by a decrease when the blend had 40% CMC content. As discussed above, at very low concentrations, polymers such as CMC are in their most extended conformation; molecules are widely separated and

Table 3.
Activation energy of compatibilized and non-compatibilized CMC/starch blends.

CMC Content (%)	[b] Activation Energy (J/mol)	[c] Activation Eneigy (J/mol)
100	15.81	9.44
80	16.72	14.92
60	17.61	19.62
50	13.40	13.37
40	8.78	19.75

the viscosity shows slight shear rate dependence Furthermore, a high loading of starch can decrease the viscosity because the starch has low viscosity than CMC.

Dynamic Tests

In Figure 1–2, the magnitudes of complex viscosity increased with CMC content and decreased with increased angular frequency. Cancela *et al.*[25] reported a similar result with blends of CMC and sucrose. The researchers observed that as shearing force increased, dynamic viscosity decreased. This was due to the orientation of the CMC molecules, as they aligned in the flow direction. Montoya *et al.*[26] also studied the rheological behavior of Arabic gum/starch blends. The viscoelastic behavior of the blends was characterized by a predominance of elastic properties. Hydrocolloids with polyelectrolytes properties, such as CMC, also have viscoelastic behavior; elasticity occurs when the polymer is a coiled chain, increasing the resistance to flow.[27]

In Figure 2, the addition of ZnO produce a decreased viscosity compared with the mixture without ZnO. The interactions between ZnO-CMC formant produce a more ordered polymer chains, facilitating the deformation of the polymer chains.

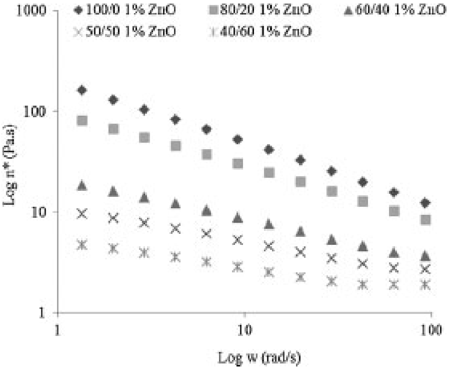

Figure 2.
Variation of the complex viscosity with angular frequency (ω) for compatibilized blends.

The phase angle (δ) may be calculated as a ratio between G''/G'. If a substance is purely viscous, the phase angle is 90°; if it is purely elastic, the phase angle is zero.[28] In Figure 3, it can be seen that as the amount of CMC in the blend increased, the phase angle magnitudes (δ) decreased. It can also be seen that as the amount of CMC increased so did the blend's elasticity. This result shows good agreement with the previously described increase of activation energy flow for the compatibilized blend. Except for the 40/60 wt% (CMC/starch), the non-compatibilized blends exhibit lower phase angle values, for the whole composition range, than the compatibilized blends. Viscous behavior increases in the presence of ZnO in the CMC/starch blends. Such behavior signals that increasing the amount of starch favors the predominance

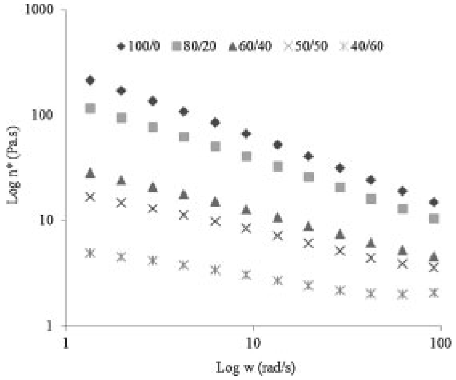

Figure 1.
Variation of the complex viscosity with angular frequency (ω) for non-compatibilized blends.

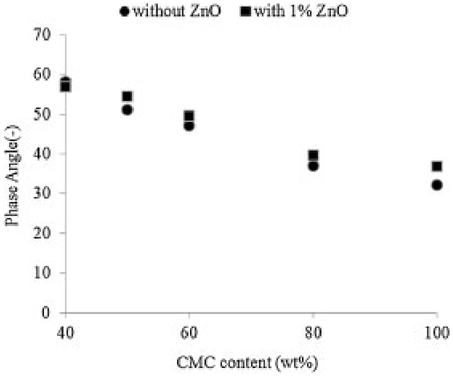

Figure 3.
Variation of the phase angle (δ) with CMC content (wt%), angular frequency (ω) 9.2 rad/s.

of viscous behavior. This is due to the increased interaction between CMC and starch chains, all promoted by the ZnO. In this case, we assume that the polymeric chains submit to an intense intermolecular attraction. This would generate less rigid and more flexible structures, dissipating more efficiently the energy applied.

Creep and Recovery Test

The viscosity zero shear rate and response time were determined through the creep and recovery test. This test, which allows us to differentiate the samples' viscous and elastic responses, introduces the stress dependence of both the viscous and the elastic behavior of solids and fluids.[29] Furthermore, tests performed in the linear region of viscoelasticity provide information about the processes of sedimentation and phase separation. The results shown in Table 4 indicate that, whenever ZnO is incorporated into the blends, a decrease results in the viscosity zero shear rate, elastic recovery, and relaxation time. Such a result suggests that the addition of zinc ions acts strongly, due to their interaction with the CMC phase, on the interactions between polymeric phases. Increasing the CMC content in the blends tends to deliver to the mixtures the stability they require to avoid phase separation. All the samples showed a progressive increase of viscosity zero shear rates.

Morphology

To investigate what interaction ZnO promoted by in the blends, we explored the morphology of CMC/starch blends. The micrographs of CMC/starch compatibilized and uncompatibilized blends can be visualized in Figure 4(a - d). For 50/50 wt% blends, the addition of 1% of the ZnO reduces the starch's average particle size and increases its dispersion in the CMC phase. We also observed the presence of co-continuous morphology. For uncompatibilized blends, starch granules were visible and the average particle size and domain of starch was greater, suggesting a poor interaction between phases. When the amount of CMC was increased, 60/40 wt% CMC/starch, seen in Figure 4(c), the addition of ZnO improved the distribution of the starch phase within a continuous phase of CMC, indicating ZnO's compatibilizing effect. We attribute the good adhesion between the phases' blend to the interaction between the carboxyl group of the CMC molecule and the zinc oxide, which improves this interaction with hydroxyl groups of starch polymers.[30–31] It is interesting to note that for 60/40% CMC/starch blends without ZnO, an increase of dispersion of the starch phases was observed. The difference on the morphology observed for both uncomaptibilized blends, Figure 4 (b) and 4 (d), is attributed to an increase in the CMC phase, which reduced the coalescence of the starch phase.

To investigate the potential use of CMC/starch as vehicles of inoculation, we carried out a survival test and compared the results with peat, a traditional vehicle used for caupi bean. Noteworthy in Table 5 is the decrease in the survival of cells for both compatibilized blends, 50/50 and 60/40 wt%

Table 4.
Creep and recovery test of compatibilized and non-compatibilized CMC/starch blends.

CMC Content (%)	a Viscosity Zero Shear Rate (Pa.s)	a Elastic Recovery (%)	a Relaxation time (s)	b Viscosity Zero Shear Rate (Pa.s)	b Elastic Recovery (%)	b Relaxation time (s)
100	7368	58.38	222.80	3904	55.54	193.90
80	1829	45.00	153.50	1451	44.18	132.10
60	118.20	23.20	84.51	86.69	14.27	56.88
50	65.63	12.38	42.37	35.20	7.85	13.63
40	5.65	1.49	6.27	13.31	2.80	11.39

aWithout ZnO;bwith ZnO

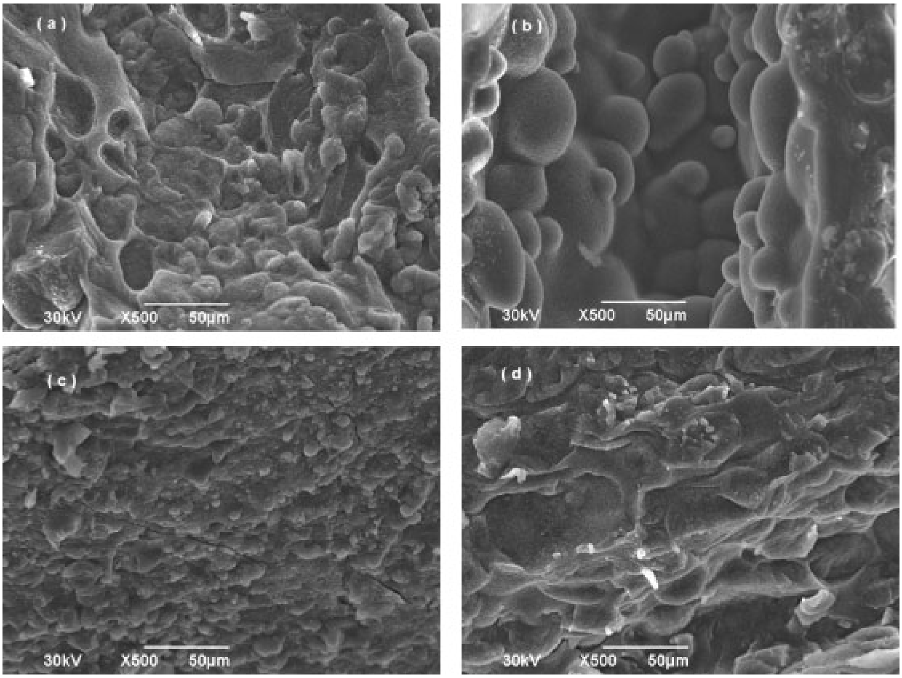

Figure 4.
SEM Micrograph of CMC/Starch blends: (a) 50/50 wt% with 1% ZnO (b) 50/50 wt% without 1% ZnO (c) 60/40 wt% with 1% ZnO and (d) 60/40 wt% without 1% ZnO.

Table 5.
Survival of Rhizobial incubated in CMC/starch blends with anda without ZnO, during 12 weeks at 26 °C.

CMC/starch Content (wt%)	Survival Log n° (cfu/mL)		
week	01	05	12
50/50 with ZnO	9.97	7.32	7.23
50/50 without ZnO	9.35	9.36	8.64
60/40 with ZnO	9.07	5.83	5.85
60/40 without ZnO	8.11	8.90	8.21
Peat	9.93	9.99	8.96

CMC/starch blends. This result can be attributed to the toxic effect of the zinc oxide on rhizobia cells. Better results of survival cells were observed for uncompatibilied blends, although better rheological results were observed for compatibilized blends.

Conclusion

This study's results showed that the addition of ZnO modifies the rheological properties of CMC/starch blends. This is a consequence of increased interaction between zinc oxide with the carboxyl group of carboxymethycellulose molecule. The addition of ZnO reduces the activation energy and the viscosity zero shear rate of the compositions, especially for those rich in starch. The creep and recovery tests indicated an increase in strain as the amount of starch and compatible mixtures increased. This indicates an increase in the viscous characteristics, except for the composition 40/60% by weight. The results of the phase angle confirmed that the addition of ZnO delivers an increased viscous phase, attributable to the interaction between the starch-CMC promoted by the ZnO. With more starch in the blend, ZnO is less effective as a compatibilizing agent on the rheological properties. This indicates that ZnO acts preferentially over CMC. Although ZnO affects the rheological properties of the blends, it harms the survival of bacteria cells. Results show that blends without ZnO have greater better potential as inoculant carriers.

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