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3D printing by additive manufacture of hydrogel containing caffeic acid

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***Keywords*—Additive manufacture, caffeic acid, hydrogel.**

***Abstract*—*In this work, 3D extrusion-based printing head was used to produce 3D geometry of hydrogel containing caffeic acid (CA). The hydrogel was prepared with 10wt.% polyvinylpyrrolidone (PVP) and 3wt.% sodium carboxymethylcellulose (CMC) using deionized water as solvent (PVP-CMC hydrogel), likewise the PVP-CMC hydrogel containing CA was prepared but was added 2wt.% CA (PVP-CA-CMC hydrogel). The rheological properties of the hydrogels were obtained using a modular rheometer with plate-plate geometry. The PVP-CMC hydrogel presented shear-thinning flow behavior and the adding of CA did not affect its rheological behavior. The power-law model described the shear-thinning curves of the hydrogels. Frequency sweep tests demonstrated that the PVP-CMC and PVP-CA-CMC hydrogels presented a physical gelation characteristic of the solid behaviors. The IR spectroscopy chemically characterized the hydrogel, indicating by carbonyl peaks a good intermolecular interaction between the functional groups. Therefore, we demonstrated the printing capacity of the PVP-CMC inks with caffeic acid, which makes it interesting to additive manufacture field.*

I. INTRODUCTION

Three-dimensional printing is a new strategy in the design market first introduced in 1980 using the technique of stereolithography [1]. Its principle of operation consists of the creation of prototypes by means of layers, where a material called ink is deposited with precision by predefined digital models [2-4]. Three-dimensional (3D) printing in the last ten years has had a constant evolution, an example of which is bioprinting, a technique in which it is linked to cell biology and materials science [5]. Compared with other manufacturing techniques where molds are required, this technique allows obtaining structures in different sizes with greater precision and at less time and cost, making it economically favorable [6].

Carboxymethylcellulose (CMC) is a semi-synthetic copolymer composed of cellulose and monochloroacetic acid in sodium hydroxide in an alcoholic medium [7]. The presence of carboxylate groups along the chain promotes the solubility of CMC in water, which, together with the low cost and advantageous properties of polysaccharides (such as biodegradability, biocompatibility, and mucous adhesion), has been used in a variety of industrial applications, as in the food, cosmetic, pharmaceutical and oil industries [8]. Comparing the mechanical properties of polyelectrolytic complexes formed by the combination of CMC with other polymers, there was an improvement in the modulus of elasticity tensile strength [9], i.e., being interesting for investigation as blends with other polymers.

Polyvinylpyrrolidone (PVP) is a polymer soluble in water and organic solvents capable of forming stable complexes with other compounds [10,11]. PVP polymer has strong hygroscopicity and can retain more than 0.5 mol of water per mol of polymer; due to its structure consisting of polar amide groups and non-polar methylene and methyl groups. For this reason, the PVP is amphiphilic. These characteristics are related to the wide application of PVP and its copolymers in the pharmaceutical industry [11,12]. Studies have shown that the use of PVP in hydrogels decreased the size of the polymeric network and the average molar mass between cross-links, and the degree of swelling in dressings [13].

Mechanical properties of the PVP are inappropriate for determining applications, i.e., has a low swelling capacity [14]. On the other hand, blended with other polymers can acquire properties interest and meet the main requirements for 3D hydrogel printing, which is structure, stability, and properties [15]. In literature, any published articles demonstrate the possibility of combining the PVP with CMC for the blend formation [14,16-18].

Caffeic acid (CA) is a phenolic compound derived from hydroxycinnamic acid and is one of the most widely distributed phenols in plant species. In addition to current medications, those that contain propolis, this acid is also present in various foods, herbs, and beverages, such as coffee, wine, apples, green tea, edible fungi, etc. [19]. In addition to having antioxidant activities, caffeic acid also has anti-cancer and antimicrobial properties, and studies show that CA has the potential to be used as a photoprotective agent when applied to skin cosmetics [19,20].

Recently, researchers [21] used an emerging technology to incorporate CA into poly (caprolactone) fibers by electrospinning. Due to the excellent antioxidant property of CA, the authors determined and suggested an ideal concentration of the incorporation of CA in PCL fibers without defective structures. The CA-loaded microfibrous PCL mat is interesting for application in the pharmaceutical and cosmetic areas.

Amorim et al. [22] approached the bioprinting technology originated from 3D printing. Normally, cells and/or bioactive compounds are added into biomaterials aiming the hydrogels formulation for 3D printing.

This work aimed to prepare the hydrogel formulation containing CA, characterize it chemically by IR spectroscopy, perform rheological testing, and evaluate its capacity of 3D printing.

II. EXPERIMENTAL

1.1 Materials

The materials used in this research were polyvinylpyrrolidone (PVP, MW = 1300000 $g.mol^{-1}$, Sigma-Aldrich), sodium carboxymethylcellulose (CMC, MW = 250000 $g.mol^{-1}$, DS = 0.7, Sigma-Aldrich), and caffeic acid powder (CA, MW = 180.16 $g.mol^{-1}$, Sigma-Aldrich). Deionized water with electrical conductivity of 0.5 $\mu S/cm^2$ was used.

1.2 Prepare of the hydrogels

All solutions were prepared using deionized water. PVP/CMC/CA solutions were prepared using 10wt.% by weight of PVP, 3wt.% by weight of CMC, and 2wt.% by weight of CA. Initially, the PVP/CA solution was prepared using a magnetic stirrer for 1 hr. CMC powder was added gradually into the solution PVP/CA over a period of 10–30 s to reduce the dispersion time, and a clear solution was obtained later of 12 hours of stirred at room temperature.

1.3 Rheological characterization

The rheological characterization was performed using an Anton Paar MCR-102 Modular Compact Rheometer using plate-plate geometry (PP50-1) with a 50 mm diameter and a gap of 1 mm to 25 °C. Measurements of the shear rate in the steady-state were performed in the range of 0.1–600 s^{-1}. Finally, frequency sweep tests were conducted in the range of 0.1–500 rad s^{-1}.

1.4 Chemical Characterization

Chemical characterization was performed by infrared spectroscopy using a spectrometer (Thermo Scientific, NICOLET iS5). All the samples were mixed with potassium bromide (KBr) in 1:100 and analyzed at 25 °C. The analysis was carried out with range from 400 to 4000 cm^{-1}, 2 cm^{-1} resolution, and 32 scans.

1.5 3D extrusion-based printing

Regarding 3D printing, the 3D extrusion-based printing head was used with the optimal PVP-CA-CMC ink. The g-code files containing the printing paths were generated using the software BioScaffoldsPG [23]. All samples were centrifuged at 4000 rpm for 5 min (Kasvi, K14-4000 model) centrifuge before printing. Then, 3D geometry of four-layers of the material were deposited (using a dosing distance of 0.2 mm, 22G needle, printing speed of 15 mm s^{-1} and a piston speed of 1.052 mm min^{-1}), and subsequently observed in a stereo microscope (Olympus, SZ-CTV model). The width of filament was measured in FIJI app. The Fig. 1 shows a schematic illustration of the preparation and the 3D printing of the 3D geometry of PVP-CA-CMC hydrogel.

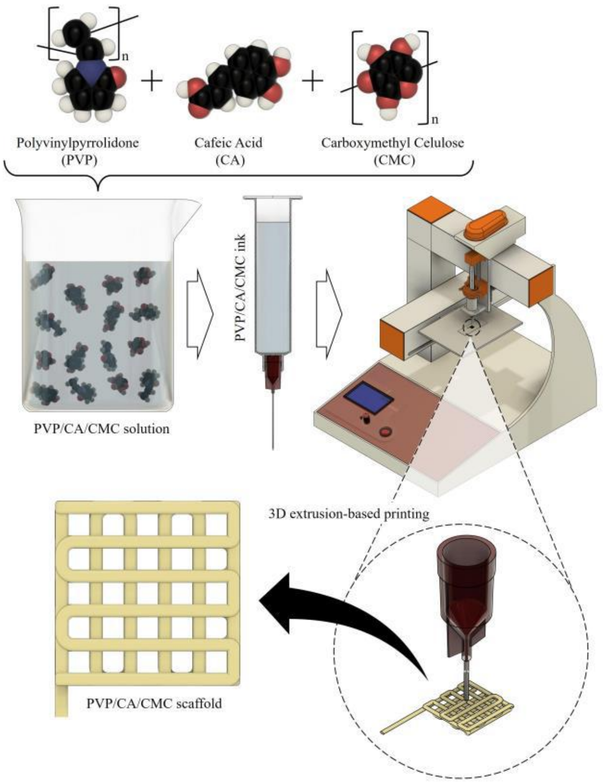

Fig. 1: Schematic illustration showing the prepare and the 3D printing of the 3D geometry of PVP-CA-CMC.

III. RESULTS AND DISCUSSION

This study shows the ability in 3D printing process of PVP-CMC hydrogel with CA from rheological and chemical characterizations. This printability was initially evaluated with steady-state viscosity and frequency rheological tests.

As seen in Fig. 2(a), viscosity decreases in solutions with and without CA as the shear rate increases. This behavior is known as shear thinning behavior and is associated with the entangling properties of polymer solutions [24]. The shear-thinning behavior is a requirement in 3D printing processes where the material, when subjected to high shear rates must flow (reduction of viscosity) and vice versa. Finally, the viscosity and the pseudoplastic behavior were not affected by the addition of CA.

The shear-thinning curves (Fig. 1) mentioned above clearly shows that the viscosity depends on the shear rate. For this reason, we used the power law model to describe this behavior by equation (1):

$$\eta(\gamma) = K\gamma^{-n} \qquad (1)$$

where $\eta(\gamma)$ is the viscosity, γ is the shear rate, K is the consistency index, and n is the flow behavior index. The fitted K and n values, including the R^2, are listed in the Table 1. Although the shear-thinning curves are similar, we observed an increases in the K when the CA to be present in the hydrogel.

Table.1: Fitted K and n values using the "equation 1", including the R^2 values.

Power-law model	***PVP-CMC Hydrogel***	***PVP-CA-CMC hydrogel***
K	*278.1*	*307.6*
n	*0.271*	*0.233*
R^2	*0.9972*	*0.9961*

Fig. 2(b) is shown the frequency sweep results used to analyze the viscoelastic behavior of the PVP-CMC and PVP-CA-CMC hydrogels. It is confirmed that PVP-CMC with or without CA hydrogels behaves mainly as a solid (G '> G") throughout the studied frequency range. This could be associated with gel formation caused by the entanglement and disentanglement of the CMC polymer chains with the PVP chains. Additionally, it was observed that the addition of CA did not significantly influence the viscoelastic properties of the PVP-CMC hydrogel.

Next, print tests were performed with the PVP-CA-CMC hydrogel. As shown in Fig. 3(a) and 3(b), it was possible to extrude the material through the nozzle and generate a printed structure which begins to collapse as the number of layers increases, but for structures that do not require large sizes it can be had considered as a printable ink and possibly applicable in areas such as medicine or in bioprinting processes. In Fig. 3(a), the filament diameter (df) was measured, obtaining a diameter of 0.638 ± 0.040mm. The diameter calculated was compared with the nozzle diameter (0.41mm). It was evidenced that the filament expanded later of extrusion due to the viscosity of the solution.

Finally in the figure 3(b), the pore diameter (dp) was also calculated with mean pore values highlighted in green circle. It was possible to observe that in the filaments where the impression began, the pore size was 0.34 ± 0.326, much smaller than the diameter presented in the center of the scaffolds. The dp of the center showed a value of 0.973 ± 0.05 and better precision and qualitative quality, being three times greater than the dp of the corners.

FTIR spectroscopy was performed to identify the functional groups that formed after the preparation of the PVP-CMC ink with and without caffeic acid and to investigate the preference for interaction of CA.

The FTIR spectra of genuine PVP and CMC polymers and the mixtures of PVP-CMC and PVP-CA-CMC hydrogels are shown in Fig. 4. For genuine CMC and PVP polymers and caffeic acid, the main vibrational bands were determined and followed by literature [25-28].

In Fig. 4, the PVP-CMC hydrogel spectrum exhibited the OH group at 3428 cm^{-1} and the carbonyl group (C=O) at 1660 cm^{-1} from the PVP; and the carboxylate group (–OOC) and (C-O-C) at 1600 cm^{-1} and 1068 cm^{-1} from the CMC, respectively. Therefore, the PVP-CMC hydrogel spectrum exhibited vibrational bands related to the functional groups of the PVP and CMC components that imply miscibility between the polymers [29,30]. The two polymers interact with each other by hydrogen bonds favoring a good hydrogel formulation.

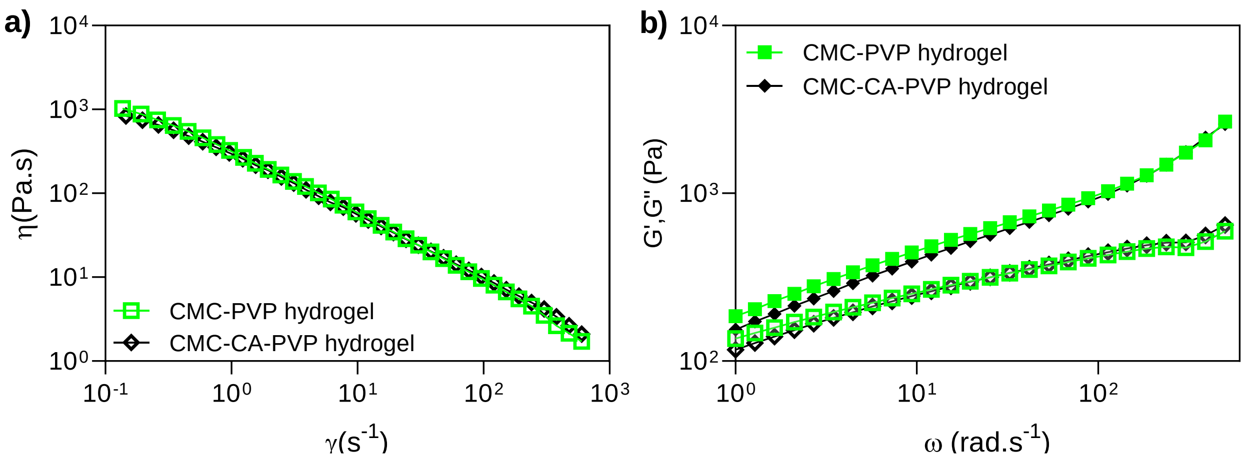

Fig. 2: Viscosity curves for CMC-PVP and CMC-CA-PVP hydrogels (a). Dependence of G' (solid symbols) and G'' (open symbols) on the angular frequency for CMC-PVP and CMC-CA-PVP hydrogels (b).

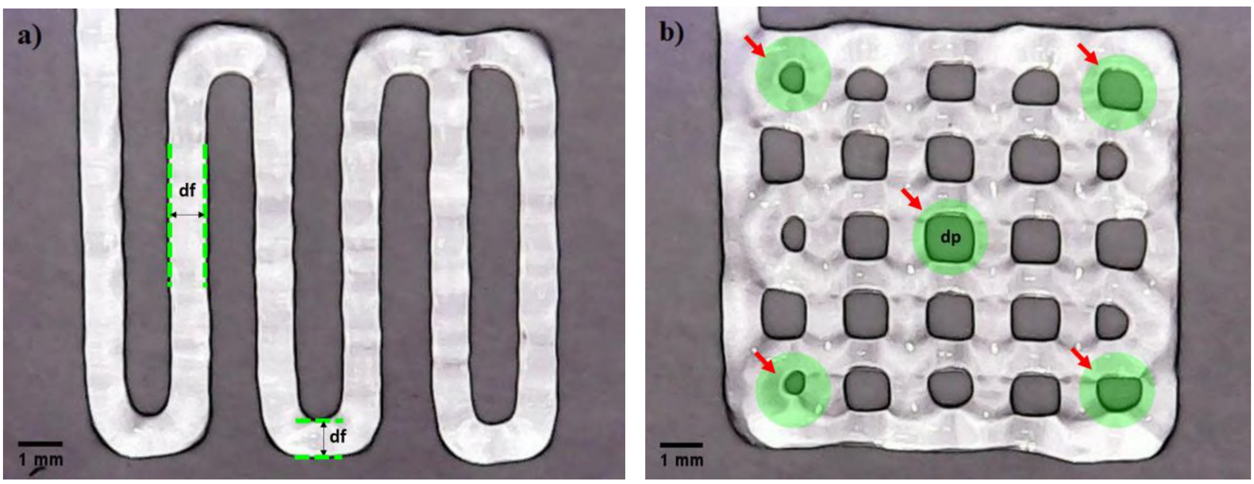

Fig. 3: Fabricated scaffold of CMC-CA-PVP for one-layer (a) and (b) four-layers, respectively, indicating the filament diameter (df) and pore diameter (dp) of the 3D geometry imprinted of CMC-CA-PVP.

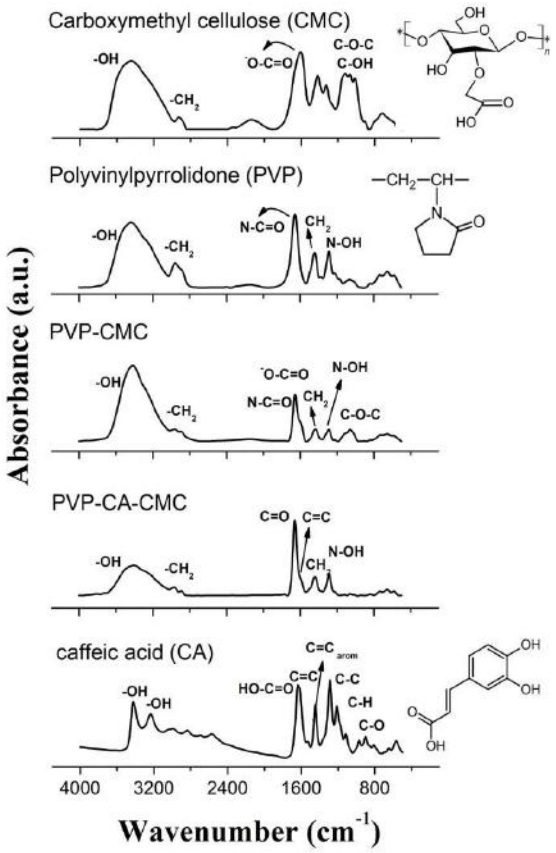

Fig. 4: FTIR spectra of CMC, PVP, PVP-CMC, PVP-CA-CMC and CA.

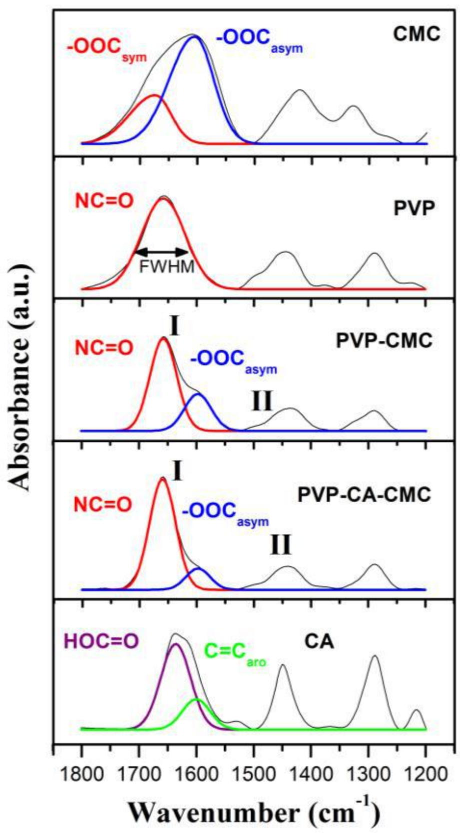

Fig. 5: Vibrational region of deconvoluted carbonyl in two Gaussian curves in the 1800 to 1200 cm^{-1} region.

Analyzing the carbonyl region in detail, Fig. 5 presents the FTIR spectra in the region from 1800 to 1200 cm^{-1} and with the bands corresponding to the deconvoluted carbonyl group in two curves. The carbonyl peaks for the PVP-CMC and PVP-CA-CMC formulations showed greater absorbency intensity and lower full width at half maximum (FWHM) values than the CMC and PVP peaks, indicating a good intermolecular interaction between the functional groups.

The ratio of peaks I/II to PVP-CMC and PVP-CA-CMC are equal to 0.76 and 0.80, respectively, showing that the presence of CA contributes to greater freedom for the movements of the PVP carbonyl and greater restriction of movement vibrational pressure for CMC carboxylate. Still for the PVP-CA-CMC hydrogel, the absorbance of the group –OOC is greatly reduced after the addition of caffeic acid, while the intensity of the vibrational band of the group C=O increases significantly. This shows that caffeic acid prefers interacting with CMC carboxylate. Furthermore, the addition of caffeic acid (CA) in the formulation of the PVP-CMC hydrogel, did not interfere with the miscibility property between polymers.

IV. CONCLUSION

3D geometry of the porous structure of the PVP-CA-CMC ink was successfully manufactured by additive manufacturing. The rheological studies allowed the analysis of the rheological properties. The addition of CA did not influence the pseudoplastic behavior of the PVP-CMC hydrogel. Frequency sweep tests demonstrated that the hydrogels with and without CA presented a physical gelation characteristic of the solid behaviors. The carbonyl peaks for the PVP-CMC and PVP-CA-CMC formulations showed greater absorbency intensity and lower FWHM values than the CMC and PVP peaks, indicating intermolecular interaction the functional groups. The printing capacity of the PVP-CA-CMC ink was demonstrated, making it attractive to additive manufacturing for 3D printing hydrogel with CA.

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