

## Supporting Information

### Poloxamer/carboxymethyl Pullulan Aqueous Systems-Miscibility and Thermogelation Studies using Viscometry, Rheology and Dynamic Light Scattering

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#### I. Structural characterization of Carboxymethyl pullulan

The degree of substitution (DS) of CMP<sub>x</sub>, which corresponds to the number of carboxymethyl groups per anhydroglucose unit, was determined by conductometric titration according to Constantin et al. [20]. For this, 0.1 g of CMP<sub>x</sub> was dissolved in 25 mL of distilled water and 5 mL of NaOH 0.1025 N were added. This solution was titrated with hydrochloric acid 0.1025 N using a conductometer CMD 210 (Radiometer, Copenhagen, Denmark) equipped with a CDC 865 cell. From the titration data, the milliequivalents of -COONa groups per gram of CMP<sub>x</sub> were calculated using Eq. (1)

$$C(\text{meq.}/\text{g}) = \frac{V_{\text{HCl}} \times c}{m} \quad (1)$$

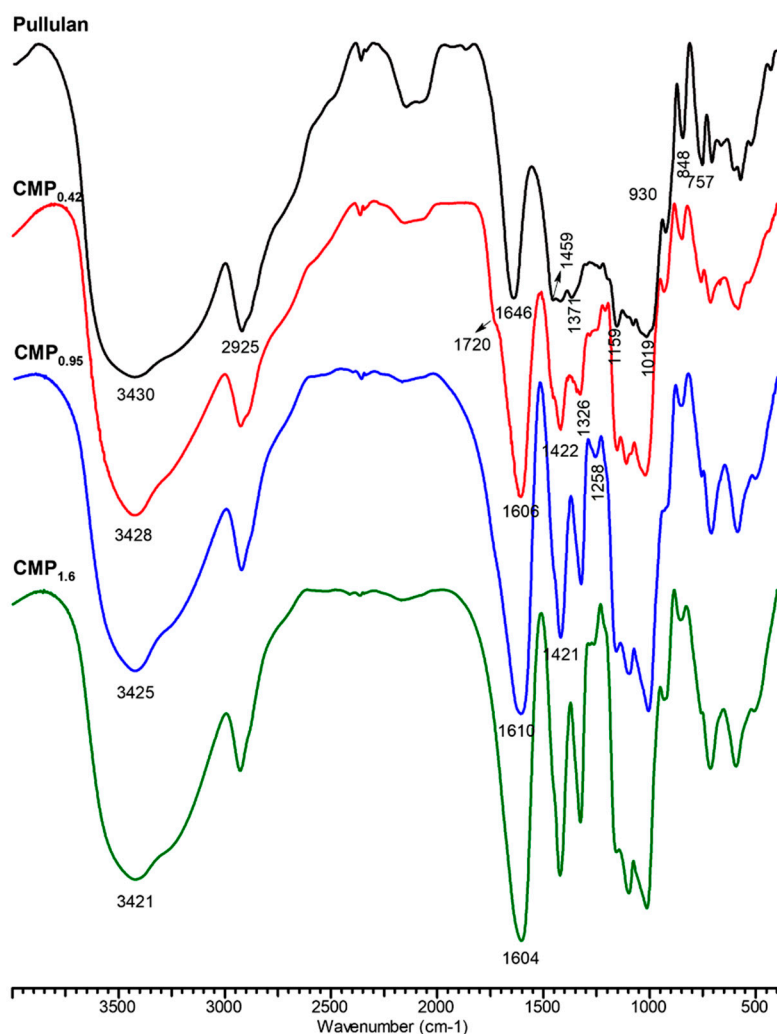
where  $V_{\text{HCl}}$  represents the volume of HCl aqueous solution determined from the conductometric titration curve,  $c$  is the concentration of HCl solution (mol/L), and  $m$  is the mass of CMP<sub>x</sub> (g) used in titration.

DS value of CMP<sub>x</sub> was determined with Eq. (2):

$$DS = \frac{162 \times C}{162 + 80 \times C} \quad (2)$$

where  $C$  represents the content of carboxymethyl groups in equivalents/gram of polymer,  $80$  is the molecular mass of the carboxymethyl radical, and  $162$  represents the molecular mass of anhydroglycosidic unit.

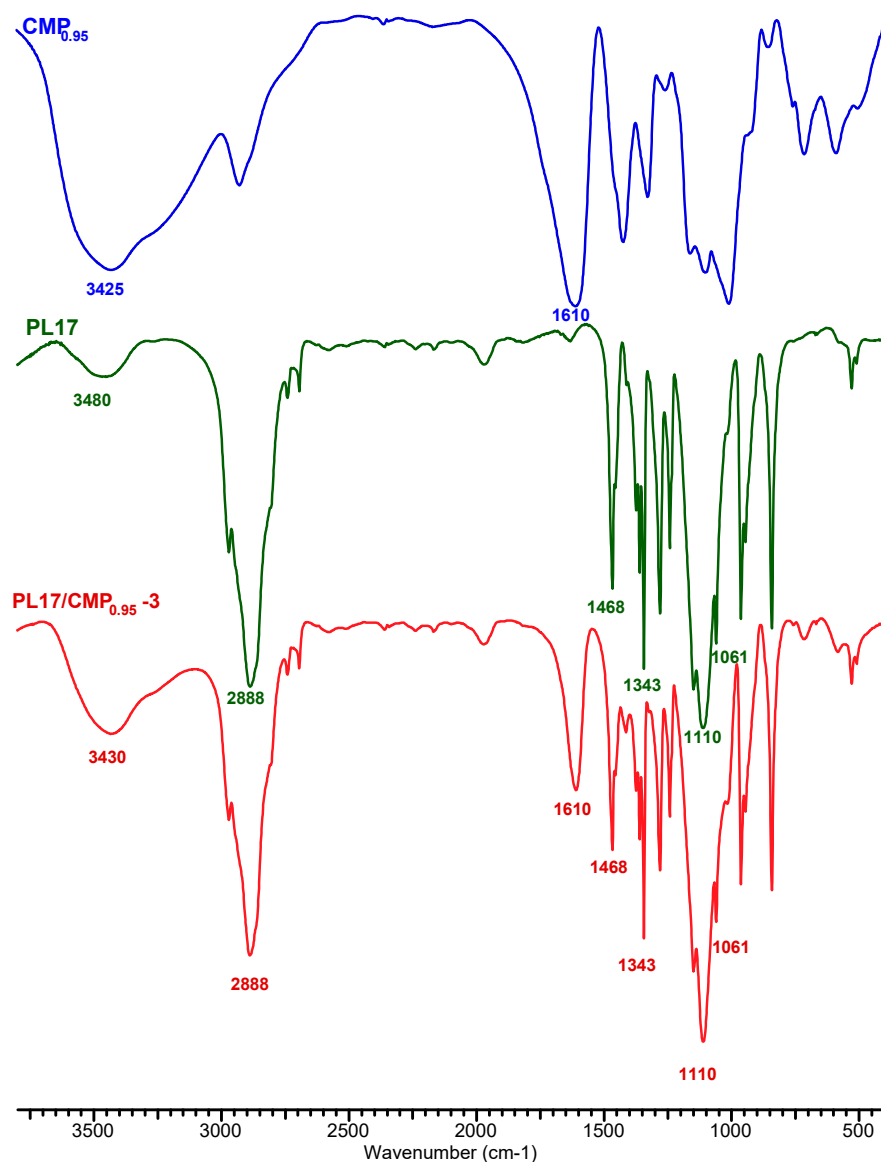
FT-IR spectra were obtained in KBr, using a FT-IR Vertex 70 spectrometer (Bruker, Austria). The spectra of native pullulan and CMP<sub>x</sub> derivatives are presented in **Figure S1**. The characteristic absorption bands of pullulan are present in the FT-IR spectrum of CMP<sub>x</sub>. In addition, the appearance of new bands specific to newly introduced carboxymethyl groups, confirms the successful realization of the carboxymethylation reaction. The absorption bands observed at  $1610 \text{ cm}^{-1}$  and  $1422 \text{ cm}^{-1}$  highlight the antisymmetric and symmetric stretching vibrations of the C=O bond from the -COO<sup>-</sup> groups [84,85]. The shoulder at  $1720 \text{ cm}^{-1}$ , corresponding to the C=O bonds from COOH groups, is visible in the CMP<sub>0.42</sub> spectrum and hidden under the large band at  $1610 \text{ cm}^{-1}$  for the others two CMP derivatives spectra, showing that the carboxylic groups are in a large extend in the form of sodium salt. The other bands were assigned as follows:  $3428 \text{ cm}^{-1}$  (O-H stretching vibration),  $2925 \text{ cm}^{-1}$  (C-H stretching vibration of methylene groups),  $1326 \text{ cm}^{-1}$  (C-O stretching),  $1157 \text{ cm}^{-1}$  and  $1020 \text{ cm}^{-1}$  (C-O-C vibration and C-O stretching bands, respectively) [86],  $852 \text{ cm}^{-1}$  corresponding to  $\alpha$ -configuration of  $\alpha$ -D-glucopyranoside units [87] and those at  $757$  and  $930 \text{ cm}^{-1}$  indicated the  $\alpha(1-4)$  and  $\alpha(1-6)$  linkages, respectively [28]. The peaks at around  $850$  and  $756 \text{ cm}^{-1}$  show the pullulan's  ${}^4\text{C}_1$  chair conformation. The reduced intensity and the shift towards to lower wavenumber of the absorption band at  $3428 \text{ cm}^{-1}$  due to the OH stretching with the increase of DS, indicates that some of OH groups were carboxymethylated. Also, with the increase in the -COONa groups number, the bands corresponding to the stretching vibration C=O of carboxylic groups increase in the intensity.



**Figure S1.** FT-IR spectra of pullulan, CMP<sub>0.42</sub>, CMP<sub>0.95</sub> and CMP<sub>1.6</sub>.

## II. FTIR spectra of PL/CMP gels

The gels obtained at 37 °C were frozen in liquid nitrogen and dried by liophilization. The samples were mixed with KBr and pelletized for FT-IR spectroscopy. **Figure S2** presents the spectra of PL17, PL17/CMP<sub>0.95-3</sub> gels together with the FT-IR spectra of the CMP<sub>0.95</sub> derivative. The characteristic peaks for poloxamer, 2888 cm<sup>-1</sup> (C-H stretching), 1343 cm<sup>-1</sup> (O-H bending), 1110 cm<sup>-1</sup> (C-O stretching), 1061 cm<sup>-1</sup> and 1468 cm<sup>-1</sup> (CH<sub>2</sub> scissor and CH<sub>3</sub> deformation vibration) [88] were present in the spectra of PL17 and PL17/CMP<sub>0.95-3</sub> samples. The presence of the band from 1610 cm<sup>-1</sup> characteristic to carboxylate groups of CMP in the spectrum of PL17/CMP<sub>0.95-3</sub> sample is an indication of the absence of chemical interaction between poloxamer and CMP. The large band from 3425 cm<sup>-1</sup> from the spectrum of CMP<sub>0.95</sub> derivative corresponding to the O-H stretching vibration was displaced at 3430 cm<sup>-1</sup> in the mixture, suggesting the formation of hydrogen bonds, most probably between the -COOH groups of CMP and the oxygens from the poloxamer chains.



**Figure S2.** FT-IR spectra of CMP<sub>0.95</sub>, PL17 and PL17/CMP<sub>0.95</sub> gels.

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