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Note

Studies of the retrogradation process for various starch gels using Raman spectroscopy

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Abstract—The retrogradation of untreated wild-type starches (potato, maize, and wheat), waxy maize starches, and one pregelatinized, modified amylose-rich starch was investigated continuously using Raman spectroscopy. The method detects conformational changes due to the multi-stage retrogradation, the rate of which differs between the starches. The pregelatinized, modified amylose-rich starch shows all stages of retrogradation in the course of its Raman spectra. In comparison to amylose, the retrogradation of amylopectin is faster at the beginning of the measurements and slower in the later stages. The untreated starches can be ranked in the order of their rate of retrogradation as follows: potato > maize > wheat.

Keywords: Starch; Retrogradation; Raman spectroscopy

Retrogradation, the characteristic behavior of starch–water systems, is the reorganization of starch molecules after heat treatment. Various methods have been used for monitoring the starch retrogradation process, such as differential scanning calorimetry (DSC),[1,2] X-ray diffraction,[3,4] nuclear magnetic resonance (NMR) spectroscopy,[5] measurements of turbidity,[6–8] viscometry,[8] and rheology,[1] as well as visualization by transmission electron microscopy (TEM).[9] Thereby, X-ray diffraction, provides evidence about crystallinity, and DSC gives information about conformational changes dependent on the temperature. Infrared spectroscopic investigations[10–14] demonstrate the possibility of using vibrational spectroscopic methods to detect conformational changes in biopolymer systems during retrogradation. Rapid-scanning Raman spectroscopy[15,16] allows continuous measurement of the process of retrogradation at the molecular level. Raman spectra of various starches have been discussed by several authors.[15,17,18]

In the literature, it is accepted that the retrogradation can be considered as a multistage process,[11,15] which includes helix formation and helix–helix aggregation. Using X-ray diffraction van Soest et al.[11] and Bulkin et al.[15] verified the results of vibrational spectroscopic measurements. Miles et al.[6,8] suggested a connection with the development of a phase separation into polymer-rich and polymer-deficient regions. Amylose and amylopectin are able to reorganize helices and crystallites.[1,19] The rate of retrogradation is increased using a lower cooling temperature and a higher difference in the temperature between heating and cooling.[16] The aggregation of amylose and amylopectin chains depends on their chain length.[19]

The objective of the study was to compare the retrogradation of various native starches and starches with different amylose and amylopectin content. Therefore, it was also interesting to investigate the retrogradation

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of the pregelatinized, modified amylose-rich starch. Raman spectroscopy was chosen to study the molecular changes and, finally, the rate of retrogradation of the various starches could be evaluated. The decreasing full widths at half height (FWHH) of Raman bands in the course of the retrogradation, as observed by Bulkin et al.,[15] were determined.

Raman spectra of the raw material maize starch, Waxilys 200, and the pregelatinized, modified amylase-rich starch are shown in Figure 1. As can be seen, there are only small differences in the spectral ranges of 400–640 and 2800–3000 cm^{-1}, which are assigned to skeletal modes of pyranose ring and to C–H stretching modes, respectively.[15] Maize starch shows bands at 478, 866, and 2909 cm^{-1}. Raman bands of Waxilys 200 are observed at 477, 867, and 2910 cm^{-1} and of pregelatinized, modified amylose-rich starch at 481, 857, and 2906 cm^{-1}. Raman bands of pregelatinized, modified amylose-rich starch of the skeletal modes and of the C–H stretching modes are wider than those of the crystalline structure, such as maize starch and amylopectin. For maize starch, Waxilys 200, and pregelatinized, modified amylose-rich starch, the FWHHs of the band at about 480 cm^{-1} are 16.2, 15.9, and 19.3 cm^{-1}, respectively, and the FWHHs of the spectral features of the C–H stretching modes between 2800 and 3050 cm^{-1} are 78.8, 81.6, and 87.9 cm^{-1}, respectively.

For the Waxilys 200 gel, Raman spectra of the skeletal and C–H stretching modes at selected time points after gelatinization are shown in Figure 2. The time evolution of FWHHs of Raman bands at 480 cm^{-1} and of the spectral feature of the C–H stretching modes

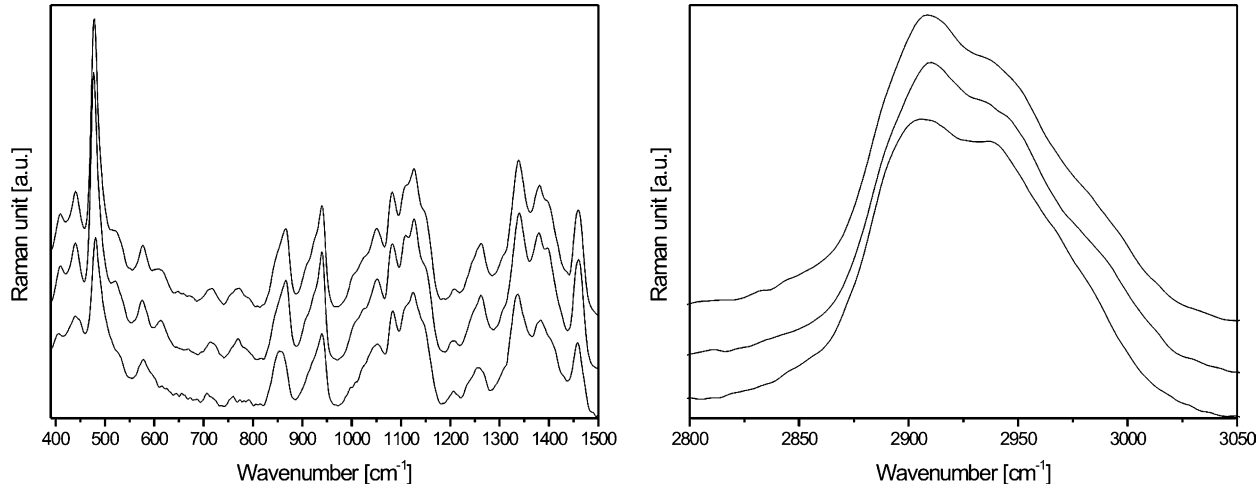

Figure 1. Raman spectra of the raw materials from top to bottom: maize starch, Waxilys 200, and the pregelatinized, modified amylose-rich starch. The spectra are normalized to the maximum of the C–H stretching modes between 2800 and 3050 cm^{-1}.

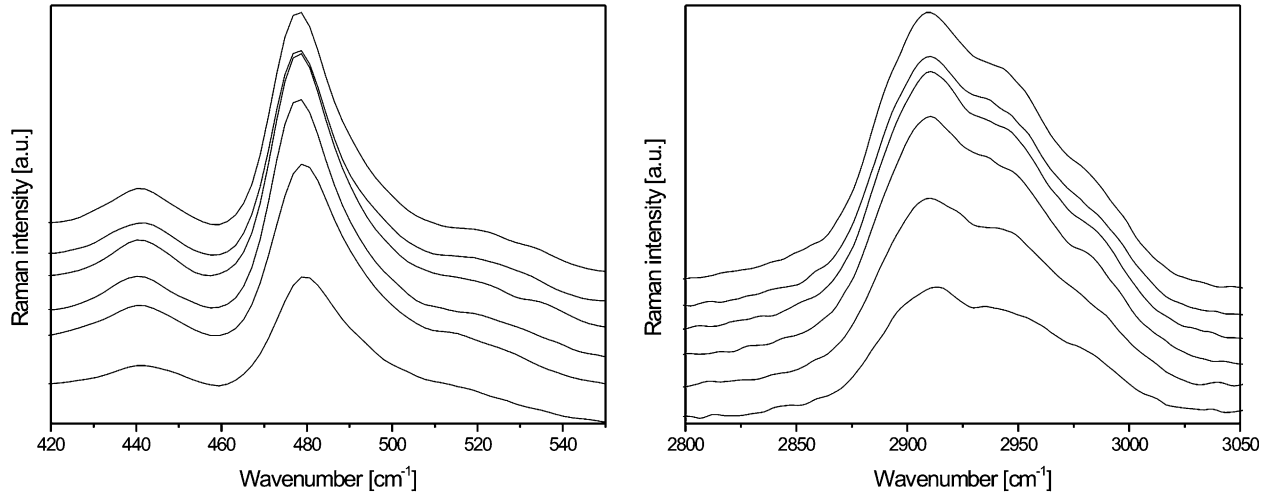

Figure 2. Raman spectra of Waxilys 200 gel at selected time points during retrogradation: Raman spectra from bottom to top after 0, 400, 800, 1000, 1200, and 1400 min.

between 2800 and 3050 cm^{-1} and the shift in the band position of Raman band at 480 cm^{-1} of pregelatinized, modified amylose-rich starch, Pregeflo C100, and Waxilys 200 are depicted in Figure 3. In the case of pregelatinized, modified amylose-rich starch (Fig. 3A), the kinetics of retrogradation observed in the range of C–H stretching modes exhibits the entire four-stage process as distinguished by Bulkin et al.,[15] namely stage I (fast), the purely conformational changes; stage II (plateau), the induction time for onset of crystal growth; stage III, the primary crystallization step, and stage IV (very slow), the crystalline phase propagation and perfection step. The pregelatinized, modified amylose-rich starch showed changes only in the C–H stretching mode; however, no changes in the skeletal modes were observed. On the other hand, Raman spectra of Pregeflo C100 (Fig. 3B) and Waxilys 200 (Fig. 3C) were sensitive to polymer conformation, which resulted in evaluable changes in the skeletal and in the C–H stretching modes. The rates of retrogradation of Pregeflo C100 and Waxilys 200 are similar to each other. In the cases of waxy maize starches, not all stages could be shown. Goodfellow and Wilson[19] report that the first step is faster for amylopectin due to its smaller helical chain lengths. This could explain why the first step was not detectable because the measuring time (400 scans are equivalent to 12 min) could be too long to register the faster first changes of amylopectin. Van Soest et al.[11] reported the differences in the entire retrogradation rate of amylose and amylopectin: the crystallization of amylose occurred within a few hours, whereas the slower crystallization of amylopectin took a few weeks.

Native starches, such as potato, maize, and wheat consist of amylose as well as amylopectin. For potato, maize, and wheat starch, the time evolution of FWHH,

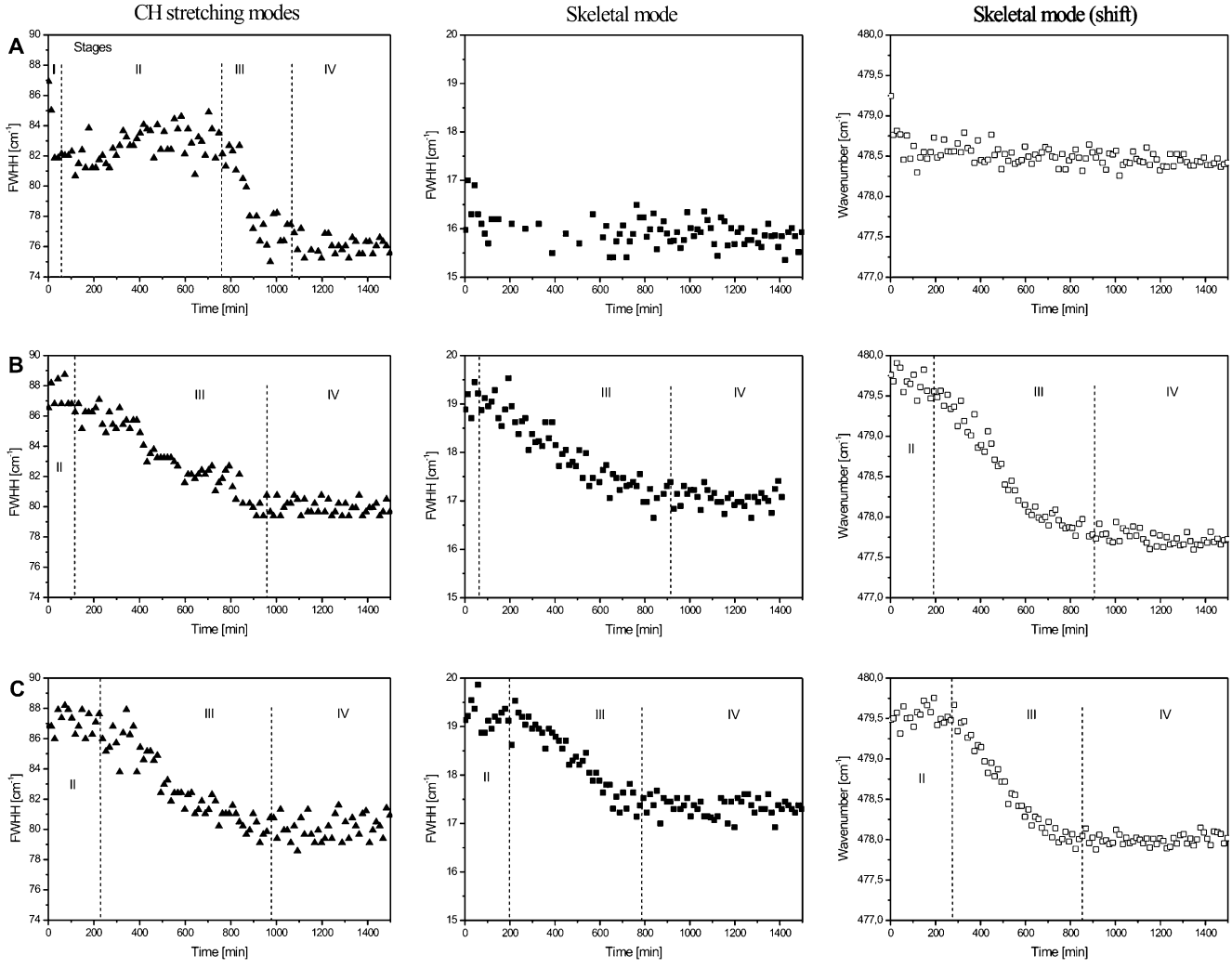

Figure 3. FWHH (▲) of the spectral feature of the C–H stretching modes between 2800 and 3050 cm^{-1}, FWHH (■) of Raman band at 480 cm^{-1}, and shifts (□) of Raman band at 480 cm^{-1} for pregelatinized, modified amylose-rich starch (A), Pregeflo C100 (B), and Waxilys 200 (C). Stages of retrogradation according to Bulkin et al.[15] are given in A.

and the shifts in the band position of Raman band at 480 cm^{-1} are shown in Figure 4. The retrogradation of potato, maize, and wheat starch could be solely observed at Raman band at 480 cm^{-1}. The retrogradation of maize starch (Fig. 4B) was slower than that of potato starch (Fig. 4A). In order to show that the process of maize starch is not finished after 1500 min, measurements were performed and evaluated up to 4000 min. The retrogradation of wheat starch (Fig. 4C) results in only a small shift of the 480 cm^{-1} band position. For the native starches, the rate of retrogradation can be qualified in the order potato > maize > wheat. These findings agree with the results of the long-term development of crystallinity of potato, wheat, and maize starch

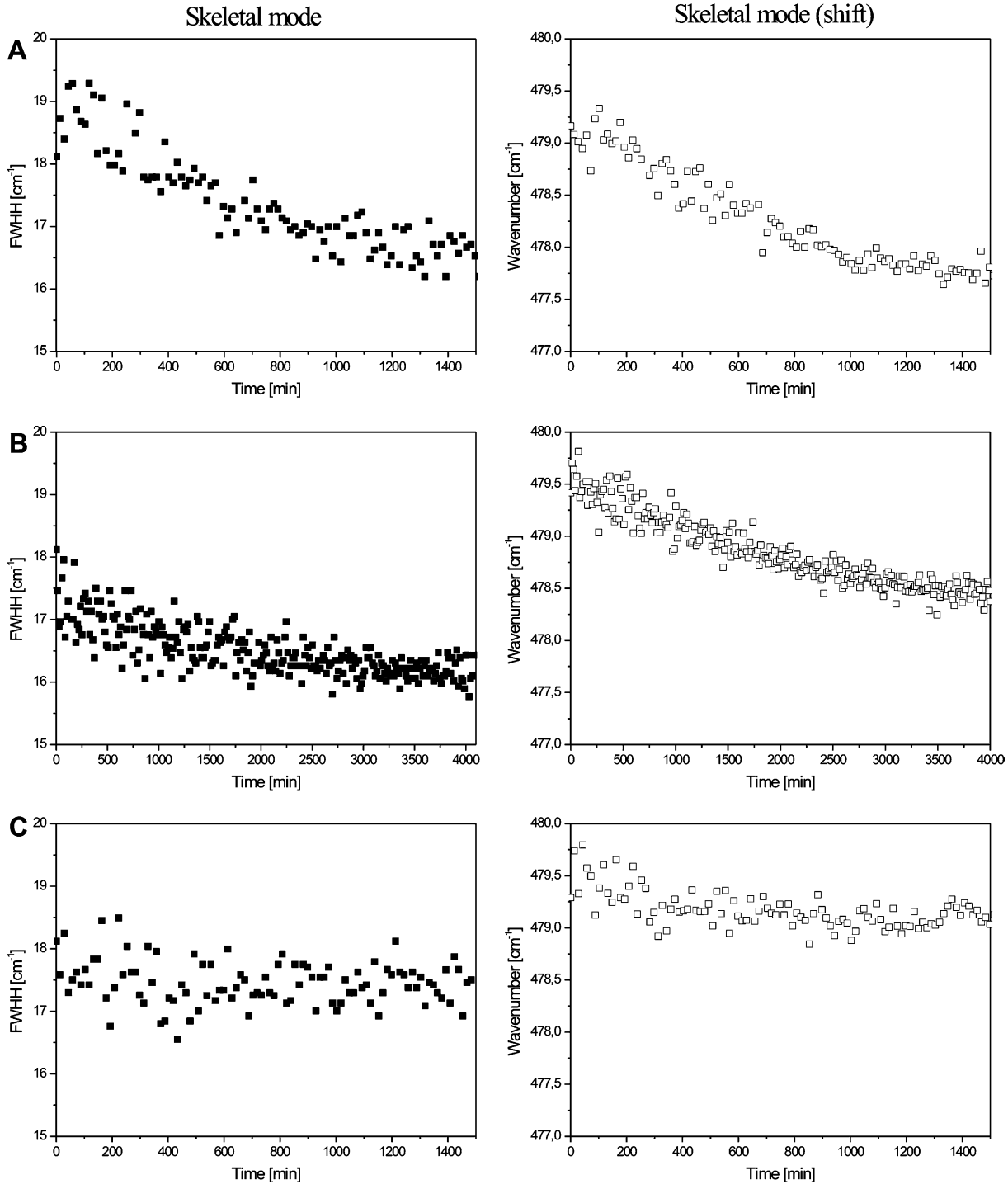

Figure 4. FWHH (■) of the spectral feature of the C–H stretching modes between 2800 and 3050 cm^{-1} and shifts (□) of Raman band at 480 cm^{-1} for potato (A), maize (B), and wheat starch (C). Note the different time scale for B.

reported by Orford et al.[20] The crystallization of amylose dominates the short-term development of the gel structure, whereas the long-term development of crystallinity is dominated by amylopectin.[3,20] On the other hand, Rein and Steffens[21] found very slow retrogradation of potato starch as determined in DSC experiments. Bulkin et al.,[15] van Soest et al.,[11] and Orford et al.[20] cooled down to a defined temperature, whereas Rein and Steffens[21] cooled down with a temperature gradient. This could lead to different conditions for the retrogradation process.

Van Soest et al.[11] and Bulkin et al.[15] observed that potato starch shows a faster retrogradation rate than waxy maize starch due to differences in the retrogradation rate of amylose and amylopectin. The authors suggested that the rapidly retrograded amylose can form an ordered matrix on a molecular level and accelerate the aggregation and crystallization of amylopectin. Our results show the fast retrogradation of the pregelatinized, modified amylose-rich starch. However, no clear differences between the rates of retrogradation of native and high-amylopectin starches were confirmed.

Raman spectroscopy as a non-invasive technique allows monitoring of the time-dependent retrogradation of starches owing to conformational changes on the molecular level. The rate of retrogradation was evaluated based on the FWHHs and the shifts in the band position of Raman bands at about 480 cm^{-1} and of the spectral feature of the C–H stretching modes between 2800 and 3050 cm^{-1}. Retrogradation is a multi-stage process differing between the various starches. The pregelatinized, modified amylose-rich starch showed the four stages, whereas in the case of the waxy maize starches, the detection of the retrogradation by Raman spectroscopy was incomplete. Potato starch, Pregeflo C100, and Waxilys 200 showed the strongest changes in the region of skeletal modes due to retrogradation. The retrogradation of the native starches was influenced by the relation of amylose and amylopectin.

1. Experimental

1.1. Materials

All starch samples were supplied by Roquette Frères (France). Demineralized water was used as the liquid. Starch products that were examined were classified as untreated starches (potato starch suprabacteriological grade, maize starch B, wheat starch TB, and waxy maize starch Waxilys 200) and starches with pre-treatment (pregelatinized, modified amylose-rich starch and pregelatinized, waxy maize starch Pregeflo C100). The amylose content of waxy maize starches is less than 5%. The pregelatinized, modified amylose-rich starch was made of high-amylose maize starch and, therefore, this starch contains about 70% amylose.

1.2. Sample preparation

Samples were prepared according to the procedure described by Bulkin et al.[15] with a concentration of 45% starch in water. Glass tubes (5 mm diameter) were used. The samples were heated for 20 min in a water bath at 90 °C in order to gelatinize the starch. Spectra were determined in the glass tubes at 30 °C. The samples showed no separation of free water.

1.3. Raman spectroscopy

Raman spectra were recorded using a Bruker FT-Raman spectrometer RFS 100/S (Bruker Optics, Germany) using a diode-pumped Nd:YAG laser at an operating wavelength of 1064 nm. The measurements were performed using the 180° angle scattering geometry with 400 scans and a laser power of 250 mW at the sample location. The interferograms were apodized with the Blackman–Harris 4-term function and Fourier-transformed to obtain spectra with a resolution of 4 cm^{-1}. The evaluation of the spectra was carried out using the Bruker OPUS software version 4.2 (Bruker Optics). Raman spectra were analyzed with respect to band position and full width at half height (FWHH) of the band of the skeletal mode at 480 cm^{-1} and of the spectral feature of the C–H stretching modes between 2800 and 3050 cm^{-1}.

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