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Short communication

EFFICIENT HETEROGENEOUS SYNTHESIS OF POLYGALACTURONIC HYDROXAMIC ACID: A VERSATILE CHELATOR FOR METAL ION BINDING

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Polygalacturonic acid, the main structure of demethylated pectin, can be efficiently transformed with hydroxylamine to the corresponding hydroxamic acids in 2-propanol under heterogeneous conditions. The degree of transformation of the carboxylic acid function can be tailored and is significantly larger than when applying a homogeneous procedure. The polygalacturonic hydroxamic acids obtained show good water solubility and are capable of forming gels with Al^{3+}, Fe^{3+} and Zn^{2+} ions. The amount of metal ions chelated by the hydroxamic acid derivative for Al^{3+} and Fe^{3+} is superior in comparison with the starting material.

Keywords: pectin, polygalacturonic acid, hydroxamic acid, metal chelate, heterogeneous synthesis

INTRODUCTION

Polygalacturonic acid (PGA), the main constituent of demethylated pectin, is often employed in the food industry as a fiber material to stabilize beverages, jams, and food emulsions.[1] More recently, the chemical modification of pectin has gained increasing interest.[2] In 2003, Hou *et al*. presented the first synthesis of a pectin hydroxamic acid in a homogeneous reaction.[3] The resulting products contained only a low amount of hydroxamic acid groups, but were active against semicarbazide-sensitive amine oxidase and angiotensin-converting enzyme. Bae *et al.* employed the same synthesis to transform pectin extracted from unshiu oranges to the hydroxamic acid derivative, still with a low degree of transformation (DT) of 10% of the carboxylic acid groups only.[4]

Hydroxamic acids play an important role in nature as ion binding and chelating agents, allowing plants the active uptake of iron ions from barren soils.[5] To test the metal binding capacity of polygalacturonic hydroxamic acids (PHA), a derivative with high hydroxamic acid content is needed to be synthesized. For this purpose, a heterogeneous synthesis protocol was employed, on the contrary to the procedures already reported. The concept of heterogeneous synthesis shows advantages over homogeneous reactions. The polysaccharide is not dissolved, so less solvent is required. Additionally, the viscosity of the reaction medium does not change during the reaction. The procedure is simple and time saving. Generally, a filtration and washing of the product is sufficient to obtain pure products. A heterogeneous synthesis protocol was already employed to convert PGA to its corresponding hydrazide quantitatively.[6] A similar procedure was applied for the conversion of PGA to its hydroxamic acid derivative. The materials obtained were analyzed by elemental analysis, viscometry, and NMR- and IR spectroscopy. Additionally, the influence of water on the conversion rate and the metal binding properties for Al^{3+}, Fe^{3+} and Zn^{2+} ions have been investigated.

EXPERIMENTAL

Materials

Polygalacturonic acid (Sigma Aldrich) had a content of galacturonic acid ≥90% (enzymatic) and Mn = 56.905 gmol^{-1}, determined by size exclusion chromatography, corresponding to a degree of polymerization (DP) of 330. Hydroxyl amine solution (50 wt% in water, Sigma Aldrich), 2-propanol (99.5%, Carl Roth), hydrochloric acid (37%, Sigma Aldrich), zinc acetate x 2 H_2O (>99.0%, Sigma Aldrich), $FeCl_3$ x 6 H_2O (99.0%, Sigma Aldrich), aluminum lactate (95%, Sigma Aldrich) and ethylenediaminetetraacetic acid disodium salt x 2 H_2O (> 99.0%, Sigma Aldrich) were used as received.

The elemental analysis was performed with a VARIO EL III CHNS analyzer (Elementaranalysensysteme GmbH). The degree of transformation (DT) is expressed in percentage and describes the amount of carboxylic acid groups that were transformed to hydroxamic acid groups in the reaction performed. The value was calculated employing the following equation:

$$DT = \frac{M_C \, x \, n_1}{M_N \, x \, n_2 \, x \, W_{C/N}} \, x \, 100 \qquad (1)$$

where DT represents the percentage of transformation of polygalacturonic acid groups to the corresponding hydroxamic acid moieties, M_C and M_N are the molar mass of carbon and nitrogen, respectively, M_C = 12, M_N = 14; n_1 and n_2 are the number of carbon and nitrogen of polygalacturonic hydroxamic acid, n_1= 6 and n_2 = 1, $W_{C/N}$ represents the mass ration between carbon and nitrogen. The equation was found in the literature[7] and modified.

Synthesis of polygalacturonic hydroxamic acid - typical example- (PHA12)

In a 100 mL round bottom flask, 2 g (11 mmol) of polygalacturonic acid (PGA) was stirred at room temperature with 5 eq. hydroxylamine solution (50 wt% in water) in 50 mL of 2-propanol for 6 hours. The mixture was filtered on a G3 glass filter and the solid material was washed twice with 2-propanol (2x 20mL). Washing once with acidified 2-propanol (100 mL with 5 ml HCl) and 10 times with aqueous 2-propanol (70%) led to a colorless solid that was dried in vacuum at 60 °C for 24 h.

Yield: 1.8 g (86%),^{1}H NMR (400 MHz, D_2O) δ: 5.07 (s, 1H), 4.44 (s, 1H), 4.00 (s, 1H), 3.76 (s, 1H); ^{13}C NMR (100 MHz, D_2O) δ: 174.6, 99.4, 78.2, 71.1, 68.7, 68.3; elemental analysis (calc. / found): C (37.70/34.01), H (4.75/5.65), N (7.33/6.03); [η]: 97.2 cm³/g; FTIR ν (ATR) [cm^{-1}]: 3154 (ν -CONH-), 2933, 2717, 1575 (ν –NH–), 1402, 1319, 1224, 1017, 1008, 949, 632.

RESULTS AND DISCUSSION

The synthesis of polygalacturonic hydroxamic acid was performed heterogeneously in 2-propanol as slurry medium. The conversion was found to be highly efficient. The reaction time, temperature, amount of water, and the equivalents of hydroxylamine were varied to investigate the influence of these reaction parameters on the conversion of the carboxylic acid group. Table 1 gives an overview of the reactions performed. At room temperature with three hours reaction time, an increase in the degree of transformation (DT) of the carboxylic acid groups with increasing amount of hydroxylamine reagent could be found. Thus, 20 eq. are required to give a product with a DT of 0.91% (PHA6), which seems to be the upper limit for this reaction. A DT of 91% can be reached with only 5 eq. reagent by increasing the reaction time to 6 hours (PHA12). An increase in temperature also improves the transformation process. At 50 °C and 6 hours reaction time, 3 eq. of hydroxylamine are sufficient to convert PGA to the hydroxamic acid form with a DT of 87% (PHA16).

Because hydroxylamine is supplied as a 50 wt% aqueous solution, the increase of the amount of reagent also increases the amount of water in the system. Thus, it influences the polarity of the slurry medium and the reactivity of the polysaccharide (PS) immersed. The effect of water on the reactivity of the PS was investigated. By keeping the amount of PGA and hydroxylamine solution constant and adjusting the volume of 2-propanol, mixtures with alcohol/water of 10:1, 4:1, and 2:1 were obtained and the results of the conversions were evaluated. The resulting DT for the three products obtained after the reactions showed to be 73%, 68%, and 40%, respectively. These findings clearly indicate that increasing amounts of water in the reaction medium decreases the reactivity of PGA. These findings could explain why the homogeneous reaction of pectin with hydroxylamine solution described by Hou *et al*. and Bae *et al*. only lead to low conversion rates.[3,4] The heterogeneous synthesis allows to tailor the DT.

FTIR spectra (recorded on a Nicolet Avatar 370DTG spectrometer using the ATR technique) of the samples with increasing hydroxamic acid content are shown in Figure 1. Starting with a DT = 8%, the valence vibration of the COOH moiety at 1728 cm^{-1} is still prominent. At higher DT of up to 91%, the intensity of this signal decreases, while two new signals at 1575 cm^{-1} and 1402 cm^{-1} become clearly observable. These new signals result from the increasing amount of

-CONH- groups formed in the product of the conversion.

Because of lack of appropriate standards, the elution volumes of the size exclusion chromatograms of some hydroxamic derivatives are given in Figure 2. Due to the heterogeneous reaction conditions employed for the transformation, only minor degradation of the polymer backbone can be observed, which results in a marginal increase of elution time for the samples with higher hydroxamic acid content.

Table 1

Reaction parameters for heterogeneous conversion of polygalacturonic acid (PGA) to the corresponding hydroxamic acid derivatives in 2-propanol with aqueous hydroxylamine solution, and the degree of transformation (DT) of the carboxylic acid group obtained

Sample	Ratio PGA/NH_2OH	Time (h)	Temp. (°C)	Elemental analysis C (m%)	H (m%)	N (m%)	DT (%)
PHA1	1:1	3	25	39.59	5.02	0.98	13
PHA2	1:2	3	25	34.27	5.41	2.51	38
PHA3	1:3	3	25	30.63	5.26	3.53	59
PHA4	1:5	3	25	35.77	5.40	5.18	74
PHA5	1:10	3	25	33.88	5.67	5.74	87
PHA6	1:20	3	25	34.93	5.53	6.20	91
PHA7	1:2	3	50	32.42	5.46	4.36	69
PHA8	1:3	3	50	31.48	5.57	5.07	83
PHA9	1:1	6	25	38.81	5.27	0.63	8
PHA10	1:2	6	25	37.77	5.26	2.87	39
PHA11	1:3	6	25	35.51	5.42	5.10	74
PHA12	1:5	6	25	34.01	5.65	6.03	91
PHA13	1:10	6	25	33.18	5.73	5.94	91
PHA14	1:20	6	25	34.65	5.65	5.96	88
PHA15	1:2	6	50	35.39	5.41	5.12	74
PHA16	1:3	6	50	34.25	5.46	5.81	87

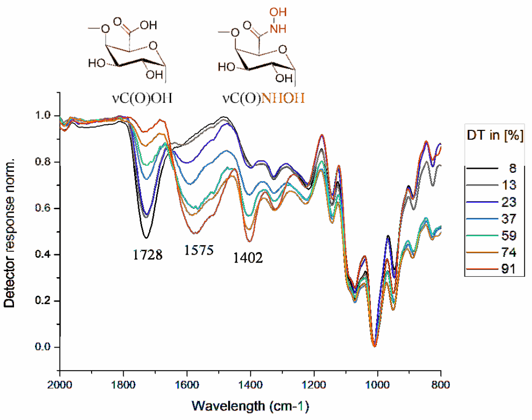

Figure 1: FTIR spectra (ATR) of polygalacturonic hydroxamic acids with increasing hydroxamic acid content

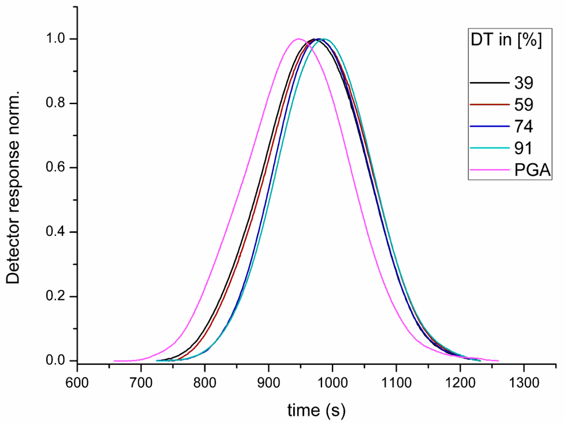

Figure 2: Elution diagram of the starting material polygalacturonic acid (PGA) and resulting hydroxamic acid derivatives with increasing DT

PGA like low methoxyl pectin is known to form gels when contacted with multivalent metal ions, such as Ca^{2+} or Zn^{2+}. To study if this property is still present and how much metal ions can be bound, polygalacturonic hydroxamic acid with a DT of 91% was dissolved in water and contacted with an excess of Al^{3+}, Fe^{3+} and Zn^{2+} ions, respectively. The three ions lead to gelation of the polysaccharide derivative. The amount of metal ions incorporated in the gels was measured in accordance to a method described earlier.[3] The hydroxamic acid derivative investigated can incorporate more than twice as much Al^{3+} ions (1.3 m%), compared to the starting PGA (0.5 m%). Fe^{3+} ions are incorporated in the hydroxamic acid derivative with 11.8 m%, which is a slightly higher amount, compared to the starting material with 9.7 m%. Considering Zn^{2+} ions, no significant differences between the hydroxamic acid derivative and the starting material could be found, with values of 12.0 m% and 11.8 m%, respectively. These results show that the transformation of PGA to the hydroxamic acid derivative is not obstructing the metal binding capability.

CONCLUSION

The heterogeneous synthesis of hydroxamic acid derivatives of PGA can be efficiently performed in 2-propanol as slurry medium at 50 °C, requiring only 3 eq. of reagent for a 91% transformation of the carboxylic acid functions. The resulting PGA derivatives are stable, water soluble, and show gel formation when contacted with multivalent metal ions (Al^{3+}, Fe^{3+} and Zn^{2+} ions). Further research will be conducted to investigate the stability of these PGA derivatives against biological degradation and their properties for metal ion filtration.

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REFERENCES

[1] C. M. P. Freitas, J. S. R. Coimbra, V. G. L. Souza and R. C. S. Sousa, *Coatings*, **11**, 922 (2021), https://doi.org/10.3390/coatings11080922

[2] H. Würfel, K. Geitel, H. Qi and T. Heinze, *Bioresources*, **16**, 8457 (2021), https://doi.org/10.15376/biores.16.4.Wurfel

[3] W.-C. Hou, M.-H. Lee, F.-L. Hsu and Y.-H. Lin, *J. Agric. Food Chem.*, **51**, 6362 (2003), https://doi.org/10.1021/jf034463a

[4] I. Y. Bae, H. J. Rha, S. Lee and H. G. Lee, *J. Excipients Food Chem.*, **2**, 89 (2011), https://ojs.abo.fi/ojs/index.php/jefc/article/view/92

[5] R. C. Hider and X. Kong, *Nat. Prod. Rep.*, **27**, 637 (2010), https://doi.org/10.1039/B906679A

[6] H. Würfel, K. Geitel and T. Heinze, *Carbohyd. Polym.*, **261**, 117838 (2021), https://doi.org/10.1016/j.carbpol.2021.117838

[7] W. Tan, Q. Li, F. Dong, S. Qiu, J. Zhang *et al.*, *Carbohyd. Polym.*, **160**, 163 (2017), http://dx.doi.org/10.1016/j.carbpol.2016.12.060