

Synthesis of Novel Polygalacturonic Acid Hydrazones and their Rheological and Emulsifying Properties

Hendryk Würfel, Gesa Pelloth, and Thomas Heinze*

Friedrich Schiller University Jena
 Institute for Organic Chemistry and Macromolecular Chemistry
 Center of Excellence for Polysaccharide Research
 Humboldtstraße 10, 07743 Jena, Germany
 E-mail: thomas.heinze@uni-jena.de

Abstract

Polygalacturonic acid, the main constituent of pectin, can be heterogeneously transformed into the hydrazide derivative. The content of hydrazide groups can be tailored easily. The water soluble and nucleophilic pectin derivative may react with long chain aliphatic aldehydes forming amphiphilic polygalacturonic acid hydrazones. Their aqueous solutions exhibit non-Newtonian flow behavior, which is shown by n -values < 1 in the Ostwald-de Waele equation. The amphiphilic polymers stabilize oil/water emulsions. As monitored by microscopy, the mixtures with oil droplets smaller than $10\ \mu\text{m}$ obtained are stable over 56 days.

Keywords: Polygalacturonic acid hydrazide, Hydrazone, o/w Emulsion, Nonanal, Pseudoplastic flow

Introduction

The research interest in polysaccharides (PS) as renewable source is strongly increasing recently. Pectin as a polyanionic representative of these class of biopolymers is especially difficult to transform completely, due to its insolubility in organic solvents. Different ways of synthesis have been reported that can increase the hydrophobic character of pectins and lead to the formation of amphiphilic materials, which can be employed as emulsifiers [1]. Zheng et al. alkylated the carboxylate group of the repeating unit with long chain alkyl halides in a heterogeneous system containing aqueous NaOH/2 propanol [2]. Furthermore, acylation reaction at the hydroxyl groups at C2, C3 have been established, using carboxylic acid anhydrides and catalytic amounts of K_2CO_3 in a solvent free reaction [3]. The treatment can be used to render pectins soluble in organic solvents for further reactions. The

procedure usually needs large amounts of organic solvent or a multistep protocol to render the samples soluble for synthesis. Recently, a heterogeneous synthesis for pectin hydrazide could be established, leading to water soluble and nucleophilic pectin derivatives very efficiently [4]. The degree of transformation of the carboxylic acid to a hydrazide group can be adjusted easily. The hydrazide formed can further react with electrophilic compounds. Thus, the question arose, if polygalacturonic acid hydrazides can be transformed to amphiphilic materials by combining the carboxylic acid group of the backbone with a long alkyl chain substituent at the hydrazide moiety. The reaction of pectin hydrazide with nonanal is reported. Infra red- and nuclear magnetic resonance spectroscopy as well as the rheological parameters and some emulsifying properties of these new pectin hydrazones are discussed.

Materials

Polygalacturonic acid hydrazide was synthesized by heterogeneous conversion of the polygalacturonic acid with hydrazine hydrate according to literature [4]. Hydrazine hydrate (80 w%) was obtained from Merck, ammonia (25 w%) from Carl Roth GmbH and nonanal ($\geq 95\%$, FCC) from Sigma Aldrich. Polygalacturonic acid (Sigma Aldrich) had a content of galacturonic acid $\geq 90\%$ (enzymatic) and $M_n = 56.905 \text{ gmol}^{-1}$ determined by size exclusion chromatography corresponding to a degree of polymerization (DP) = 330. All other reagents were of analytical grade and have been used without further purification.

Methods

Fourier Transform Infrared (FTIR) spectra were recorded on a NICOLET AVATAR 370 DTG spectrometer using translucent KBR tablets. The NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer at 40 °C. The sample concentration was at least 60 mgmL⁻¹. Deuterium oxide and deuterated dimethyl sulfoxide (dms_o-d₆) was used for the measurements. Samples were referenced to 3-(trimethylsilyl)-propane-sulfonic acid sodium salt. Size exclusion chromatography (SEC) was carried out with an Agilent 1200 Series LC equipment (SEC pump G1310A, RI detector G1362A, columns: PSS Gram30 and PSS Gram1000 in series, flow rate: 1 mL min⁻¹; eluent: H₂O / 0.08 M Na₂HPO₄ / 0.05% NaN₃ pH 9. The calibration standard was polyethylene glycole (PEG). A VARIO EL III CHNS analyzer (Elementaranalysensysteme GmbH) was used for elemental analyses. Flow curves have been obtained on a Haake Mars Modular Advanced Rheometer System. The temperature was controlled within 0.1 °C with a Haake UTM Controller. A microscope Axioskop 40 Zeiss was used to investigate the droplet sizes of the emulsions. All pictures have been taken with a Canon EOS 1300 D. The images were analyzed with the software ImageJ.

Emulsions have been formulated by combining the aqueous polymer solution with sunflower oil and mixing the compounds for 60 seconds at 4000 min⁻¹ with a T25 Ultra Turrax. The samples were stored in a refrigerator at 7 °C or in a laboratory-type drying cabinet at 25 °C and 60 °C.

Synthesis

Polygalacturonic acid hydrazide was synthesized according to Literature[4]. In a typical procedure 5.0 g (28 mmol) polygalacturonic acid was suspended in 100 mL 2-propanol. Hydrazine hydrate (1.8 g, 56 mmol) was added, and the mixture was allowed to stir at room temperature for two hours. The solid was filtered, acidified with 50 mL 2-propanol/HCl conc. (95 vol%/5 vol%) and subsequently washed with 2-propanol/H₂O (80 vol%/20 vol%) 10x 50 mL. The yellow solid was dried in vacuum at 60 °C for 24 h. Yield: 4.92 g (99%)

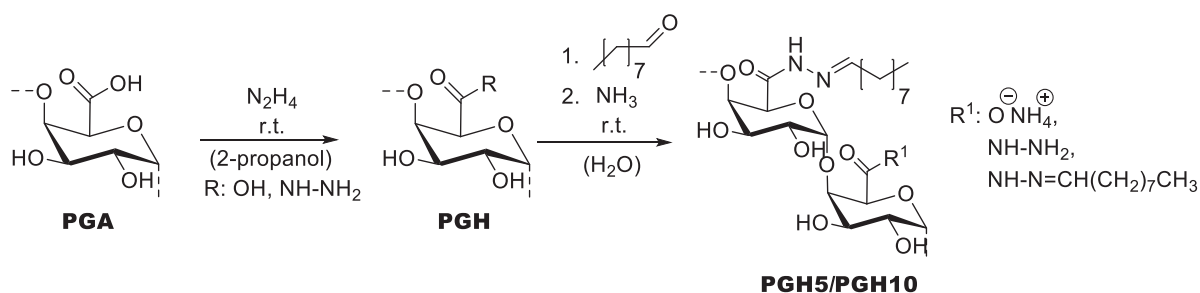
FTIR (KBr) ν [cm⁻¹]: 3186 (v, OH), 1723 (v, COOH), 1579 (v, C(O)NH-), 1402, 1232, 1088, 1066, 1007, 944, 624. ¹H NMR (400 MHz, D₂O, ppm) δ : 5.05, 4.80, 4.40, 3.95, 3.74 (repeating unit). ¹³C NMR (100 MHz, D₂O, ppm) δ : 174.7 (C6), 99.4 (C1), 78.3, 71.2, 68.8, 68.3. Elemental analysis (m%) (calc./found): C (37.9/ 35.2), H (5.3/ 5.7), N (14.7/ 1.0), hydrazide content: 7%.

In a typical procedure for the hydrazone formation, 2.0 g (11 mmol) polygalacturonic acid hydrazide was dissolved in 50 mL deionized water and 1.0 g (7 mmol) nonanal was added with stirring at room temperature. After 5 min 10 mL of conc. ammonia was added and the mixture was poured into ethanol (300 mL), the precipitating solid was filtered on a G3 glass filter, washed 3 times with ethanol (200 mL) and twice with ethylacetate (200 mL). The product was dried in vacuum at 60 °C for 24 h. Yield: 1.93 g (95%).

FTIR (KBr) ν [cm⁻¹]: 3220 (v, OH), 1726 (v, COOH), 1590 (v, C(O)NH-), 1413, 1224, 1088, 1074, 1013, 949, 630. ¹H NMR (400 MHz, D₂O, ppm) δ : 7.10, 5.01, 4.04, 3.76, 3.62, 1.26, 0.85. ¹³C NMR (100 MHz, D₂O, ppm) δ : 175.2, 102.4, 80.9, 73.0, 70.7, 66.9, 63.4, 60.1, 34.4, 31.8, 26.4, 25.2, 19.5, 16.4. Elemental analysis (m%) (calc./found): C (37.9/ 38.4), H (5.3/ 5.9), N (14.7/ 0.8).

Results and Discussion

The heterogeneous synthesis of reactive polygalacturonic acid hydrazides could be employed to prepare derivatives with low content of hydrazides in a tailored fashion [4]. A material with hydrazide content of 7% has been synthesized heterogeneously in 2-propanol. It was subsequently allowed to react with nonanal for 5 min and 10 min, which resulted in the pectin hydrazones **PGH5** and **PGH10** (Scheme 1).



Scheme 1. Starting from polygalacturonic acid (PGA) a heterogeneous synthesis leads to polygalacturonic acid hydrazide (PGH), which was reacted for 5 min and 10 min in water with nonanal to form polygalacturonic acid hydrazones (samples **PGH5** and **PGH10**)

The hydrazones formed have been neutralized with ammonia to increase their water solubility by forming the ammonium polygalacturonate. The materials thus

obtained show different colors as depicted in Figure 1. The materials were dissolved in dist. water and their rheological behavior was characterized.

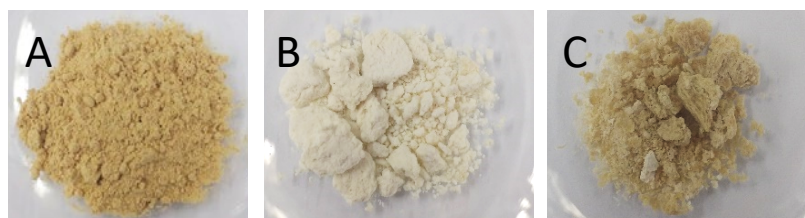


Figure 1. Appearance of the starting material polygalacturonic acid (A) and the final polygalacturonic acid hydrazones **PGH5** (B) and **PGH10** (C).

Rheological behavior

The flow curves of the pectin, pectin hydrazide, and the hydrazones **PGH5** and **PGH10** investigated show that all samples behave as non-Newtonian fluids (Figure 2). Their apparent viscosity decreases with increasing shear rate. This pseudo-plastic behavior is very pronounced for the polygalacturonic acid hydrazide in the range of shear rate from 1 s⁻¹ up to 100 s⁻¹. The reorientation of the macromolecules along the direction of flow and the disentanglement of the molecule chains can explain these findings.

A general trend of decreasing apparent viscosity with increasing temperatures at shear rates above 100 s⁻¹ can be seen for all samples. This temperature-viscosity behavior is especially seen in the range from 20 °C to 40 °C and less pronounced from 40 °C up to 60 °C for pectin, **PGH5** and **PGH10**. The PGH samples investigated show an increase in viscosity with increasing temperature at low shear rates (< 20 s⁻¹). At higher shear rates (> 20 s⁻¹), the viscosity decreases with increasing temperature. This effect is much less pronounced than for the other samples investigated.

The shear dependence of the viscosity can be further analyzed considering the Ostwald-de Waele power-law (Equation 1):

$$\tau = k \cdot \dot{\gamma}^n \quad (1)$$

Here τ is the shear stress, k is the consistency index and n is the power-law exponent [5]. For shear thinning fluids $0 < n < 1$ and for shear thickening fluids n is larger than 1. Plotting both the shear stress and the shear rate logarithmically, values for n and $\log k$ can be obtained (Figure 3). All obtained values are given in Table 1.

Figure 3 shows the linear graphs for $\dot{\gamma} > 30$ s⁻¹. Therefore, equation 1 can be employed. It was found that the n -values are lower than 1 in the temperature range investigated (20 °C to 60 °C). This indicates indeed shear thinning behavior for all samples in the shear rate range considered. Polygalacturonic acid shows only a small change in n -value from $n = 0.94$ (20 °C) to $n = 0.93$ (60 °C). On the contrary, pectin hydrazide shows a clear decrease in n -value at increasing temperatures. Starting with the highest value $n = 0.87$ at 20 °C the exponent decreases to $n = 0.77$ at 40 °C and to $n = 0.71$ at 60 °C. The two pectin hydrazide derivatives **PGH5** and **PGH10** are showing significant different n -values. With increasing temperature, **PGH5** shows n -values of $n = 0.78$, $n = 0.83$ and $n = 0.77$ at 20 °C, 40 °C and 60 °C. For **PGH10** the n -values are comparable with the starting pectin ranging from $n = 0.97$, $n = 0.98$ and $n = 0.80$ at 20 °C, 40 °C and 60 °C, respectively.

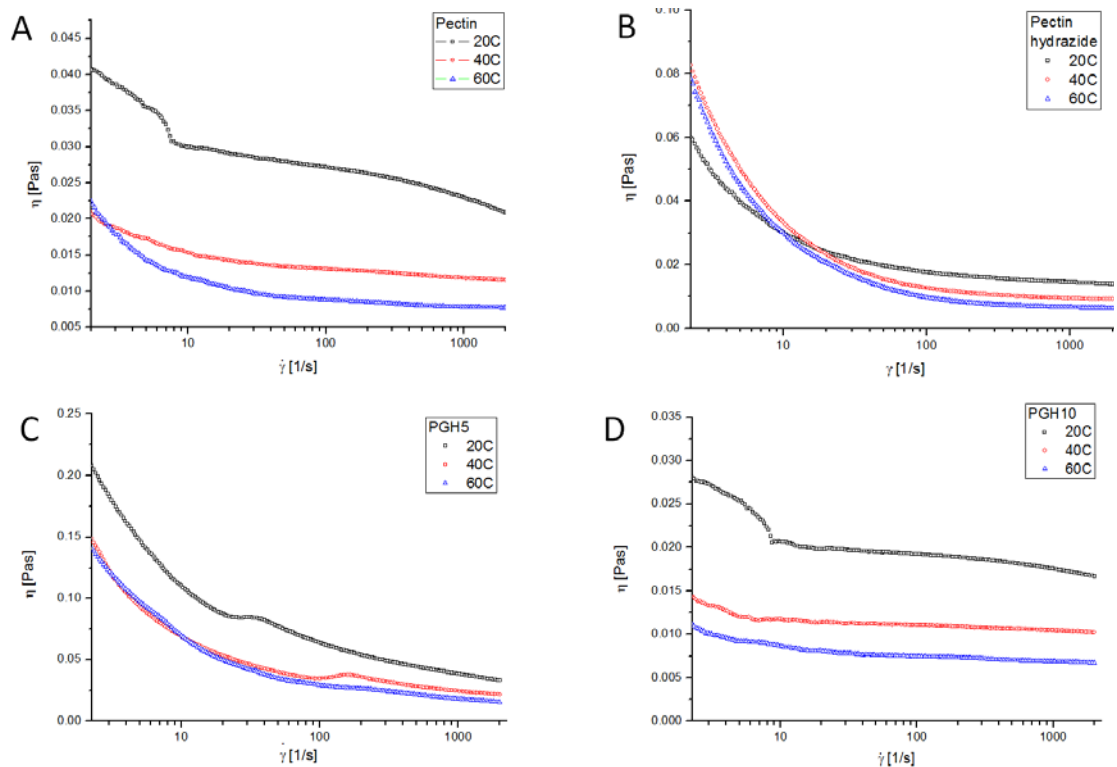


Figure 2: Apparent shear viscosity versus shear rate of pectin (A), pectin hydrazide (B), PGH5 (C) and PGH10 (D) in aqueous solution at 20 °C, 40 °C and 60 °C respectively.

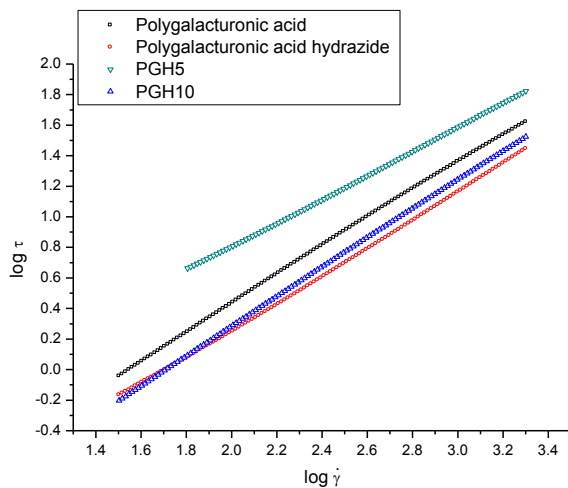


Figure 3: Logarithm of shear stress versus logarithm of shear rate of polygalacturonic acid, polygalacturonic acid hydrazide, PGH5 and PGH10 at 20 °C.

Emulsifying properties

The polygalacturonic acid hydrazide derivatives **PGH5** and **PGH10** synthesized show good water solubility. Thus, samples with 2.5 m%, 5 m%, and 10 m% polysaccharide derivative have been dissolved in deionized water and mixed with sunflower oil (up to 14 m%). The emulsions formed have been stored at different temperatures (7 °C, 25 °C and 60 °C) over a period of 56 days. The diameter change of the oil droplets over time was monitored with a microscope and photographs were taken to measure the size of the emulsified oil droplets. The samples stored at 60 °C separated within minutes after the storage temperature was reached. The samples stored at r.t. or 7 °C showed a clear trend. Samples with lower amount of PGH derivative separated faster than samples with higher mass% PGH derivative.

Table 1: Rheological parameters of aqueous solutions of polygalacturonic acid, polygalacturonic acid hydrazide, PGH5 and PGH10.

	T = 20 °C		T = 40 °C		T = 60 °C	
	<i>n</i>	<i>k</i>	<i>n</i>	<i>k</i>	<i>N</i>	<i>k</i>
Pectin	0.94	0.035	0.95	0.017	0.93	0.012
Pectin hydrazide	0.87	0.034	0.77	0.041	0.713	0.043
PGH5	0.78	0.179	0.83	0.083	0.77	0.090
PGH10	0.97	0.022	0.98	0.012	0.80	0.020

Table 2: Mass ratios of polygalacturonic acid hydrazone derivatives with 14 m% sunflower oil and the time dependent change in diameter of the produced emulsion droplets after mixing. Samples have been stored at 7 °C.

	PGH Derivative	Mass %	Droplet Diameter [μm]	
			Start	After 56 d
EM1	PGH5	2.5	8	23
EM2	PGH5	5.0	9	10
EM3	PGH5	5.0*	7	9
EM4	PGH10	5.0	7	8
EM4	PGH10	10.0	5	6

* Sample was mixed with 5 m% Glycerin

A picture of emulsion EM2 stable for the time of investigation (56 days) and the relative occurrence of the size of emulsified oil drops is shown in Figure 4. The influence of glycerin as a stabilizer was tested in one trial as well (EM3) but gave only marginal changes in the outcome of the droplet size and stability of the emulsion formed. Other additives for stabilization of the emulsion e.g. against microorganism growth

have not been tested. It could be observed that a few samples started to mold after a few days, which lead to the destruction of the emulsion within a single day. These samples have been prepared again and showed to be stable on the second trial. No correlation could be found between the appearance of mold and the composition of the emulsion.

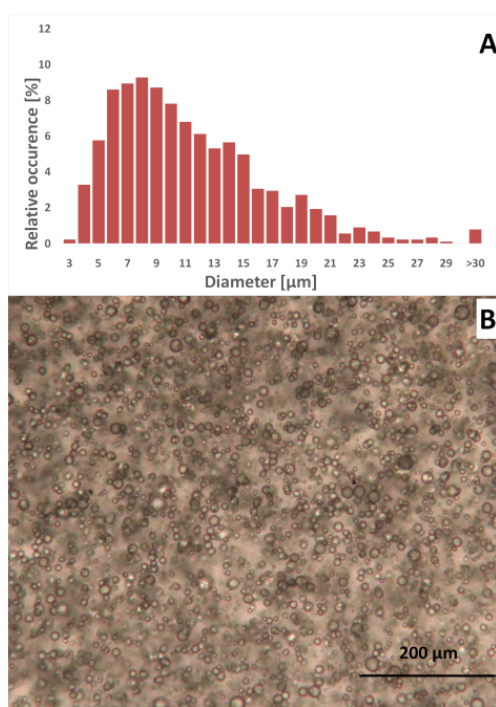


Figure 4: Relative occurrence and diameter of emulsified oil droplets of sample EM2 (A) after 56 days of storage and (B) a magnified view of the same sample.

Conclusions

Polygalacturonic acid hydrazide, as one of few nucleophilic polysaccharides, can be synthesized with a tailored hydrazide content. The nucleophilic group formed can be employed to synthesize hydrazones. Conversions with the hydrophobic aldehyde nonanal and subsequent neutralization of the carboxylic acid groups with ammonia lead to the formation of amphiphilic materials. The polymers investigated show non-Newtonian behavior. This is most evident for PGH. In contrast to the starting PGA and the final materials **PGH5** and **PGH10**, the PGH shows the smallest change in the apparent viscosity during measurement with shear rates above 100 s^{-1} . The apparent viscosity of PGH at low shear rates ($< 10 \text{ s}^{-1}$) even shows a temperature-viscosity behavior reversed compared to the other samples investigated. The amphiphilic materials **PGH5** and **PGH10** show emulsifying properties and stabilize oil in water emulsions over several weeks with droplet sizes as low as $6 \mu\text{m}$ after 56 days of storage at $7 \text{ }^\circ\text{C}$. Pectin hydrazide can function as a platform for new and interesting pectin hydrazones, shifting from petrochemical based to more sustainable resources. Future studies will show, how the stability of these amphiphilic materials can be increased over a larger temperature range. Moreover, the introduction of further functional groups via this path is under investigation and will expand the applicability of pectin and its derivatives.

References

- [1] H. Würfel, K. Geitel, H. Qi, and T. Heinze, Chemical Modification of Pectin and Polygalacturonic acid: a critical review. *Bioresources*, 16, 8457-8488, (2021).
- [2] X. F. Zheng, Q. Lian, H. Yang, D. D. Jia, and D. J. Wang, Synthesis and characterization of alkyl-pectin materials. *Asian J. Chem.*, 25, 2121-2124, (2013).
- [3] L. Monfregola, M. Leone, V. Vittoria, P. Amodeo, and S. D. Luca, Chemical modification of pectin: environmental friendly process for new potential material development. *Polym. Chem.*, 2, 800-804, (2012).
- [4] H. Würfel, K. Geitel, and T. Heinze, Efficient heterogeneous synthesis of reactive polygalacturonic acid hydrazides. *Carbohydr. Polym.*, 261, 117838, (2021).
- [5] K. Lewandowska, A. Dąbrowska, and H. Kaczmarek, Rheological properties of pectin, poly(vinyl alcohol) and their blends in aqueous solutions. *e-Polymers*, 12, 015, (2011).