Green Synthesis of Hyperbranched Polyglycerol at Room Temperature

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Supporting Information

ABSTRACT: In this work we report on a new method for the cationic polymerization of glycidol by citric acid at ambient and solvent free conditions. In this polymerization, citric acid is a proton donor and is able to incorporate in the structure of polyglycerol by reaction with the activated monomer. The molecular weight and degree of branching of the synthesized polymers are affected by the glycidol/citric acid molar ratios and reaction temperature. Due to the citric acid core of the hyperbranched polyglycerols, they are able to break down into the smaller segments at neutral or acidic conditions. Apart from citric acid, glycidol, and water, other reagents or organic solvents have not been used in the synthetic and purification processes. Taking advantage of the green synthesis and ability to cleave under physiological conditions, in addition to the intrinsic biocompatibility of polyglycerol, the synthesized polymers are promising candidates for future biomedical applications.

In recent years, hyperbranched polyglycerols (hPG) have been used in a wide variety of applications due to their thermal stability, biocompatibility and low toxicity, originating from three-dimensional branched structure and high number of end-hydroxyl groups.1−5 In general, hPGs are synthesized by cationic or anionic ring-opening polymerization of glycidol.6−21 In the anionic process, strong bases are necessary to deprotonate the initiator and start the polymerization process.22,23 Therefore, synthetic processes should be performed in aprotic solvents and dry conditions. Additionally, temperatures as high as 100 °C, long reaction times, and special polymerization methods are required to produce hPGs with desired molecular weights.11 However, anionic polymerization is the only method, so far, by which polyglycerols with the desirable molecular weights could be synthesized.11

On the other hand, cationic ring opening polymerization of glycidol in buffer solution or using Lewis acids catalysts are the alternative methods for the production of hPG and its copolymers under mild conditions.12−15 The products of these polymerization methods are of high interest due to their food, personal care, and industrial applications.16 Therefore, different reaction parameters affecting the structure of the synthesized polymers through this method are studied and investigated.14,15,17 However, control over the molecular weight and structure of the synthesized polymers, which is an advantage for the anionic ring opening polymerization of glycidol, is not developed in the case of cationic method.

Hence, developing the new methods for polymerization of glycidol at ambient conditions using a green chemistry approach is necessary for the low-cost and high-scale synthesis of the highly pure hPGs with the desirable molecular weight and structure. Low biodegradability of hPG is another issue that should be addressed for in vivo biomedical applications. In this respect, several successful experiments have been performed to improve the biodegradability of hPGs by incorporation acetal, ketal, ester, and disulfide bonds into the polymer backbone.18−22

Citric acid (CA) is supposed to be a proton donor23,24 and suitable activating agent for the cationic ring opening polymerization of glycidol under mild conditions. It is a cheap, commercially available, and biodegradable compound. It is known as intermediate in the metabolism and can be found almost in all animals and plants especially in citrus fruits.25 It is widely used in the food and pharmaceutical industries as well as detergent, cleaner, and cosmetic products.26 Citric acid has been also used as reducing and capping agent for the synthesis and stabilization of metal nanoparticles.27 Dendrimers and copolymers of citric acid have shown potential biomedical applications.28−30

Herein, we report on a solvent free method for the cationic polymerization of glycidol by citric acid at ambient conditions in a short time reaction. The structure of the synthesized polymers are affected by the molar ratios of glycidol/citric acid ([G]/[CA]).

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Citric acid with three pKa ranging from 3.13 to 6.4 is able to activate glycidol toward the cationic polymerization. This result is expected, because carboxylic acids are reported to be able to protonate glycidol and therefore induce its cationic polymerization. However, spectroscopy data show that citric acid is a part of the synthesized polymers and its function is more than a proton donor. In order to investigate the role of citric acid, a series of polymerizations using different \([\text{G}]/[\text{CA}]\) ratios were performed. Polymers synthesized by 5/1, 15/1 and 30/1 molar ratios of \([\text{G}]/[\text{CA}]\), are nominated as hPG5, hPG15 and hPG30, respectively. Figure 1 shows the IR and \(^1\)H NMR spectra of the synthesized hPGs. The absorbance band of carbonyl groups in the IR spectra and the proton signals of citric acid in the \(^1\)H NMR spectra of different products clearly prove the presence of citric acid in the structure of hPGs.

Increasing the intensity of the carbonyl bands, in the IR spectra, as well as peak area of signals of citric acid in the \(^1\)H NMR spectra with decreased \([\text{G}]/[\text{CA}]\) ratio suggest that citric acid is a potential initiator in the polymerization process (Figure 1). MALDI-TOF MS and \(^1\)H NMR spectroscopy shows that the molecular weight of hPGs is slightly affected by the \([\text{G}]/[\text{CA}]\) ratio (Figures S6–S8 and Table 1). These results confirm that the citric acid is participating in the polymerization of glycidol. Moreover, the mass distribution of the synthesized polymers follows \(189 + 74n\), where \(n\) is the number of glycerol units and 189 is the mass of citric acid segment. Two-dimensional
heteronuclear multiple-bond connectivity (HMBC) NMR shows a correlation between the protons of glycerol units at 3.7−4.2 ppm and two signals of the carbonyl groups of citric acid segment at 170−180 ppm (Figure 2).

Therefore, it can be concluded that citric acid is in the core of hPG. While the ability of carboxylic acids or carboxylates to initiate the polymerization of glycidol has been proved previously,31−33 we performed a control reaction between trisodium citrate and glycidol to evaluate the efficiency of carboxylate groups for the opening of the glycidol ring. NMR spectra show that the product of reaction consists of citric acid and glycerol blocks (Figures S1 and S2). In the ESI-MS spectrum of the product of this reaction, peak at m/z 189 is assigned to citrate ions (Figure S3). This peak is followed by other peaks at m/z 189 + 74n, where n is the number of glycidol units. These results prove that carboxylate groups of citric acid are able to initiate the polymerization of glycidol.

There are two proposed mechanisms for the cationic polymerization of glycidol in literature:6,8 activated chain end mechanism (ACE) and activated monomer mechanism (AM). Inverse gated 13C NMR spectra showed that synthesized polymers are containing L1,4 units in addition to L1,3 units (Figure 1c). Since the relative abundance of the L1,4 structural unit is high in the polymer backbone, we conclude that the mechanism of polymerization follows the activated monomer (AM) mechanism.6,8,15 Therefore, the proposed mechanism for the polymerization of glycidol by citric acid at room temperature includes the protonation of glycidol and ring opening of the activated epoxides in the focal point of oligomers by carboxylate groups (Figure 3).

In this mechanism, carboxylate groups are able to open the activated epoxide ring of either monomers or oligomers, which are growing by autopolymerization. However, the citric acid content of polymers is lower than that in the [G]/[CA] feed ratios. This result shows that a major part of citric acid is not incorporated in the polymer structure and it is only consumed for

<table>
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<th>structural units</th>
<th>shift (ppm)</th>
<th>hPG5</th>
<th>hPG15</th>
<th>hPG30</th>
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<tr>
<td>T_2</td>
<td>82.5−83.5</td>
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<tr>
<td>2L_1,3</td>
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<td>L_1,3, L_1,4</td>
<td>62.0−63.0</td>
<td>1.26</td>
<td>1.77</td>
<td>2.77</td>
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</table>

reaction time (min) | 15 | 180 | 600 |
deregion of braching | 0.54 | 0.36 | 0.32 |
terminal units (%) | 20 | 26 | 25 |
dendritic units (%) | 30 | 16 | 15 |
linear 1,3 units (%) | 25 | 30 | 35 |
linear 1,4 units (%) | 25 | 28 | 25 |

M_n (MALDI) | 1026 | 1286 | 1411 |
M_w (MALDI) | 1118 | 1489 | 1660 |
PDI (MALDI) | 1.08 | 1.15 | 1.17 |
M_n (NMR) | 1092 | 1430 | 1630 |
PDI (GPC) | 1.39 | 1.72 | 1.82 |
yield (%) | 50 | 55 | 73 |
the protonation of monomers. This part of citric acid is removed upon purification.

The degree of branching (DB) which is an indicator for the tree-like structure of polymers was calculated by the equation introduced by Frey et al. While this equation was initially applied for hPGs synthesized through anionic polymerization, it has been successfully used in the case of cationic polymerization. According to this equation, DB of polymers was in Figure 3. Schematic representation of the proposed mechanism for the ring opening polymerization of glycidol by citric acid. Citric acid plays the role of both proton donor and initiator in this polymerization.

Figure 4. $^1$H NMR spectra of (a) hPG, and the products of its degradation and purification at (b) pH 5 and (c) pH 7.4. (d and e) $^1$H NMR spectra of triethyl citrate and sodium citrate, respectively. Shift or decrease of the peak area of citric acid signals (spectra b and c) show the hydrolysis of ester bonds. Some of citric acid units are separated from the polymer upon hydrolysis at pH 5 and therefore they are removed after purification process. However, at neutral pH ester bonds are partially hydrolyzed and citric acid units cannot be removed by dialysis. In pH 7.4, the hydrolyzed bonds are present in the form of citrate, and therefore, signals of citric acid block shift toward lower fields, partially. The $^1$H NMR spectra of triethyl citrate and sodium citrate are also shown as controls.
the range of 0.32–0.54, which is higher than reported data for the cationic polymerizations\textsuperscript{14} and close to that for the anionic opening polymerization.\textsuperscript{34} This is due to the relative abundance of the structural units of the synthesized polymers. While the amount of dendritic and L\textsubscript{1,3} blocks are close to the reported values for the cationic polymerization of glycidol,\textsuperscript{14,15} the relative abundance of L\textsubscript{1,4} blocks is lower than that and this results in a higher DB for the synthesized polymers (Table 1).

Moreover, the relative abundance of the dendritic units (D\%) and also DB of hPGs depend on the [G]/[CA] ratio, inversely. This is due to the activation of a larger number of glycidol monomers increases the possibility of a reaction between the secondary hydroxyl groups and those monomers.

Polydispersity index (PDI) of the synthesized polymers was determined by MALDI-TOF MS and gel permeation chromatography (GPC) (Figure S10). It was found that PDI values are depended on the [G]/[CA] ratios and the lower amount of the citric acid resulted in the higher PDI (Table 1). Polymerizations were performed in multigram scale and the yield of reactions was typically in the range of 50–73\% (Table 1). Reactions of the synthesis of hPG\textsubscript{5}, hPG\textsubscript{15} and hPG\textsubscript{30} were terminated after 15, 180, and 600 min, respectively, because the viscosity of the product was high and stirring was not possible anymore. In order to study the effect of the temperature of reaction on the structure of polymers, hPG\textsubscript{5} was synthesized at 20, 40, and 80 °C and products were analyzed. It was found that rising the temperature of the reaction increases the rate of polymerization, drastically (Table S2). Also, DB, D\%, and relative abundance of the L\textsubscript{1,3} and L\textsubscript{1,4} was altered by changing the temperature of reaction (Table S2).

Ester bonds between citric acid core and polyglycerol branches should induce cleavage of hPGs to the smaller segments in the physiological conditions. Although the molecular weight of the synthesized polymers is low and their degradation at the present form is not significant, it will be crucial when they are used for the construction of bigger objects such as nanogels.

In a typical experiment, hPG\textsubscript{5} was stirred in PBS solutions with pH 5 and 7.4 at 37 °C for 7 days, and then it was purified by a dialysis bag with the molecular weight cut off 100–500 g mol\textsuperscript{-1}. At pH 5, the intensity of the carbonyl band in the IR spectra and signals of citric acid in \textsuperscript{1}H and \textsuperscript{13}C NMR spectra decreased (Figures S14b, 4b, and S15b). These results show that the ester bonds between citric acid unit and polyglycerol branches are hydrolyzed and some of citric acid molecules are removed upon the purification process (Figure 4b). In contrast, under neutral condition (pH 7.4) signals of protons of citric acid in \textsuperscript{1}H NMR spectra shift from 2.8–3 ppm to 2.4–2.6 ppm, partially (Figure 4c). Furthermore, in the IR spectra, a new absorbance band for the carbonyl group of citric acid is appearing at 1585 cm\textsuperscript{-1} (Figure S14c). These new signals and absorbance bands in \textsuperscript{1}H NMR, IR, and \textsuperscript{13}C NMR spectra are assigned to the citric acid units that are in the form of citrate (Figures 4e, S14e, and S15e). At neutral conditions, ester bonds are hydrolyzed, partially, to produce hPGs with the citrate focal point. As a result the hydrolysis and degradation of polymers depend on the pH of medium and lower pH accelerates this process. Moreover, MALDI-TOF and GPC measurements of degradation products without purification showed reduction in the molecular weight which is due to the breaking of molecules to smaller segments (Figures S17 and S18). HMBC NMR of degraded products showed no correlation between the citric acid carbonyl group and polyglycerol protons which is due to separation of citric acid and polyglycerol parts (Figure S16). DOSY NMR measurement of hPGs showed a similar diffusion coefficient for citric acid core and hPG branches. However, after degradation, the diffusion coefficient of the citric acid segment was twice as high as that of the hPG branches. This result proves that citric acid and hPG segments are separated upon degradation (Table S3).

In summary, citric acid is an efficient initiator and proton donor for bulk polymerization of glycidol at ambient conditions. Citric acid plays the key role of both proton donor and initiator in the polymerization process. The molecular weight and degree of branching of the synthesized hPGs as well as the citric acid content of polymer are slightly affected by the [G]/[CA] ratio. The synthesized hPGs are hyperbranched in nature and they can break down into smaller branches under neutral or acidic conditions. Since this method is based on a green chemistry approach, products are free from impurities and suitable for cosmetic, biomedical, and industrial applications.

**ASSOCIATED CONTENT**

**Supporting Information**

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Experimental section, along with supporting figures, tables, and references (PDF).

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**Notes**

The authors declare no competing financial interest.

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