

Cross-linking of γ -Cyclodextrin Using Non-toxic Polyethylene Glycol Spacer Units

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Abstract: The crystalline cyclic oligosaccharide γ -cyclodextrin (γ CD) is a natural, non-toxic and biodegradable precursor that can be used to prepare innovative polymers. We cross-linked γ CD with variable quantities of non-toxic polyethylene glycol diglycidyl ether (PEGDGE) in aqueous NaOH to form 3D structures with tunable physicochemical properties. Mixtures with a 5:1 PEGDGE: γ CD molar ratio produced a cross-linked material that was soluble in water and organic solvents, underwent glass transition at -23°C . The properties of this material were further modified by additional curing with PEGDGE or nitration reaction. Curing produced an insoluble polymeric material with excellent swelling abilities (200% in water) and decreased glass transition temperature to -37°C . The nitrated cross-linked γ CD was soluble in common organic solvents and underwent glass transition at $+19^\circ\text{C}$, achieving thermal stability below 190°C . These novel γ CD polymers are promising materials for applications in the pharmaceutical and food industries and environmental remediation.

Several types of polymers such copolyesters, styrenes, polyurethanes, polyamides and polyolefin blends,^[1-3] can be cross-linked with bifunctional or multifunctional molecules such as isocyanates, polyols or diazides to create toughened 3D networks with a variety of properties.^[4-9] Naturally occurring materials such as proteins and carbohydrates can also be used in cross-linking reactions producing derivatives for various types of applications.^[10-12] For example, cyclodextrins (CDs) are crystalline cyclic oligosaccharides produced naturally by the enzymatic degradation of starch.^[13-19] Alpha (α), beta (β) and gamma (γ) CDs (Figure 1) are the most common forms, containing 6, 7 and 8 glycoside units, respectively, and differing in the size of the central cavity: 5.7 Å for α CD, 7.8 Å for β CD and 9.5 Å for γ CD.^[13,20]

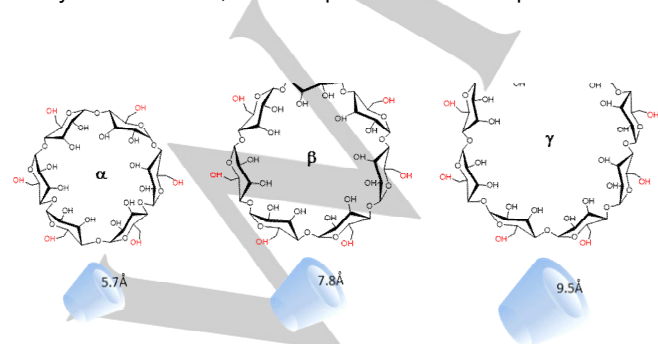


Figure 1. Chemical (top) and toroidal (bottom) structures of α , β and γ cyclodextrins.

α , β and γ CDs were first cross-linked using epichlorohydrin, and different degrees of cross-linking were achieved by altering the reaction environment.^[21] Varying the type and length of the cross-linker,^[10,22-24] influences the solubility and thermal and mechanical properties of the polymeric CDs, with shorter links producing brittle materials characterized by high glass transition temperatures ($T_g > 0^\circ\text{C}$) but longer links yielding softer materials with glass transition occurring below -10°C . The length of the cross-linker also affects the solubility and swelling of the cross-linked polymers.^[25] In our previous studies, β CD was cross-linked with non-toxic diglycidyl ethers to produce soluble cross-linked polymeric materials and their nitrated derivatives.^[8,26-27] These materials displayed good thermal and thermo-mechanical properties, with glass transitions occurring at temperatures as low as -17°C and showed promising inclusion and compatibility properties for pharmaceutical applications.^[28-30] CDs and CD derivatives are widely used for the solubilization and stabilization of water-insoluble chemical substances.^[31-32] CDs are also used in the development of polyrotaxanes by forming supramolecular macrocyclic polymers promoters of guest-host interactions.^[33-35] The present study reports the synthesis and characterisation of a new family of polymeric materials soluble in water and organic solvents and suitable for further chemical and physical modification, using γ CD as a precursor.

The cross-linking of γ CD with PEGDGE involved an S_N2 nucleophilic substitution and was based on our earlier reported procedure for the synthesis of cross-linked β CD.^[8] Samples of the γ CD were first dissolved in aqueous NaOH and left to stir mechanically (160 rpm) at room temperature for 24 hours followed by dropwise addition of excess cross-linker (5:1, 6:1 and 7:1 PEGDGE: γ CD molar ratios) (SI, experimental section). The reaction mixture was then stirred (330 rpm) at 30°C , for 5-22 h or until the formation of a very viscous phase (Figure 2).

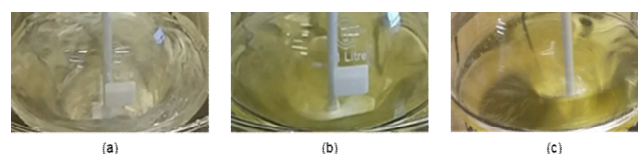


Figure 2. Synthesis of γ CPCDs at time 0 h, (b) 3 h and (c) 6 h. The viscosity of the reaction mixture increases with the time.

After neutralization and precipitation in excess acetone to remove unreacted and/or degraded cross-linkers, the precipitated solid was dissolved in distilled water and dialysed against water

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for 4 days using a benzylated cellulose membrane with a molecular weight cut-off of 2000 Da. The volume of the dialysed solution (lacking NaCl as a reaction by-product, unreacted γ CD and low-molecular-weight oligomers < 2000 Da) was then reduced and the concentrate was dried under high vacuum to produce pure cross-linked cyclodextrin samples (γ CPCD) (Table 1), with a typical yield of 75%.

Sample	PEGDGE: γ CD molar ratio	Reaction time (h)	PEG: γ C D unit ratio	$T_g^{[1]}$ (°C)	$T_m^{[2]}$ (°C)	$T_c^{[3]}$ (°C)
γ CD	-	-	-	+115	320	340
γ CPCD-1	5:1	6.00	3.3:1	-19	248	256
γ CPCD-2	5:1	22.00	3.7:1	-23	247	255
γ CPCD-3	6:1	5.35	3.8:1	-19	248	255
γ CPCD-4	7:1	4.55	3.7:1	-22	270	276
γ CPCD-5	5:1	6.00	3.7:1	-23	247	255

Table 1. Influence of the PEGDGE: γ CD molar ratio on the degree of cross-linking (PEG: γ C D unit ratio) and thermal transitions of the corresponding γ CPCD products.

[1] From the mid-point of the third heating cycle in DSC experiments

[2] Onset of melting point determined by DSC

[3] Onset of mass loss determined by TGA

The solubility of the γ CPCDs was assessed in a variety of common organic solvents and it was found that this material was soluble in water, methanol, ethanol, dichloromethane and chloroform but insoluble in acetone, ethyl acetate and hexane. The NMR spectrum of γ CPCD-1 (5:1 PEGDGE: γ CD molar ratio) in DMSO- d_6 showed broadened peaks compared to the γ CD and PEGDGE precursors (Figure 3), confirming that the macromolecular structure of the cross-linked product represented a complex chemical environment with overlapping protons. (SI, Table S1).^[8,27]

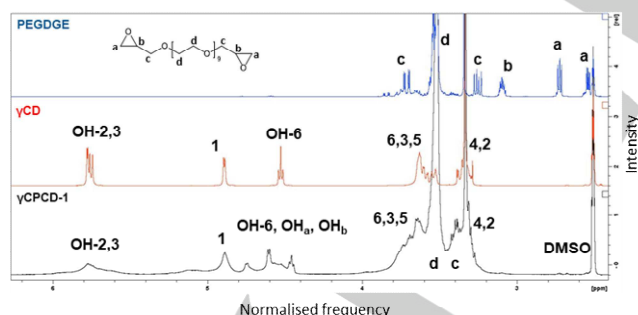


Figure 3. ^1H NMR spectrum of PEGDGE (Top), γ CD (Middle) and γ CPCD-1 (Bottom).

The methylene (CH_2) protons attached to carbon atoms c and d of the PEG spacer units, the methine (CH) protons attached to carbon atoms 1, 2, 3, 4, 5 and 6 of the γ CD units, and the OH-2 and OH-3 protons attached to carbon atoms 2 and 3 of the γ CD units, were identified in the cross-linked material. Cross-linking was confirmed firstly by the consumption of the CH_2 and CH protons attached to carbon atoms a and b of the PEGDGE epoxy ring, and secondly by the reduction of the integrals from OH-6 protons attached to carbon atom 6 of the γ CD units at 5.9 ppm (indicating formation of new ether linkages in the region between 5.0 and 4.6 ppm). The macromolecular structure was also

confirmed by the formation of a new broad peak centred at 5.10 ppm assigned to the absorption of the anomeric methine CH-1 protons attached to carbon atom 1 and adjacent to a substituted proton in the OH-2 group (Figure 4, where $\text{R} = \gamma$ CD, PEG spacer, or PEG entanglement).

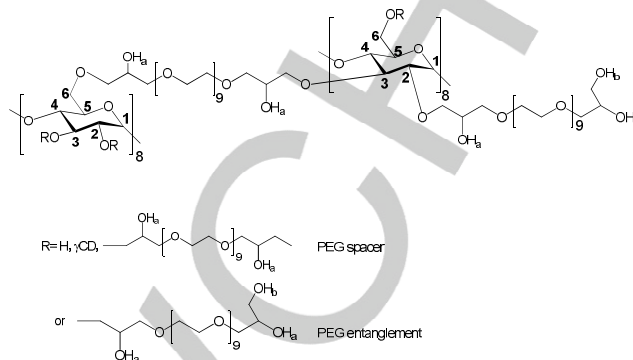


Figure 4. Proposed chemical structure of γ CPCDs with numbered carbon atoms of γ CD units.

The degree of cross-linking (expressed as the PEG: γ C D unit ratio) in the γ CPCD samples was determined by ^1H NMR spectroscopy in D_2O (SI, Figure S1),^[8] and was estimated to be 25% lower than the theoretical maximum ratio that can be achieved based on the PEGDGE: γ CD molar ratio. This reflected the degradation of the cross-linker under alkaline hydrolysis conditions. The γ CPCD samples were also compared to the γ CD and PEGDGE precursors by FTIR spectroscopy (SI, Figure S2). The strong absorption at 2940 cm^{-1} (symmetric stretching of CH_2 and CH), 2871 cm^{-1} (asymmetric stretching of CH_2 and CH), 1453 cm^{-1} (scissoring of CH_2), 1350 and 1249 cm^{-1} (bending CH), and 1080 and 1024 cm^{-1} (stretching of C-O-C) confirmed the presence of cross-linking in γ CPCD.

Sample γ CPCD-1 with a 3.3:1 PEG: γ C D unit ratio calculated from ^1H NMR spectrum, was synthesised using a 5:1 PEGDGE: γ CD molar ratio and an optimal reaction time of 6 h (Table 1). Increasing the PEGDGE: γ CD molar ratio, generated additional cross-links, with a molar ratio of 6:1 leading to a PEG: γ C D unit ratio of 3.8:1 (γ CPCD-3) and a molar ratio of 7:1 leading to a PEG: γ C D unit ratio of 3.7:1 (γ CPCD-4), respectively (Table 1). However, the outcome was also dependent on the duration of the reaction time. For samples γ CPCD-3 and γ CPCD-4, the reaction was stopped before the optimum 6 h (5.35 h and 4.55 h, respectively) due to earlier formation of hydrogel, resulting in a comparable degree of cross-linking. In addition, the characterisation of the pre dialysed γ CPCD-4 sample by ^1H NMR in D_2O highlighted the increasing prevalence of non-reacted PEGDGE at higher molar ratios. Although hydrogel formation had started, not all the cross-linker was used up, suggesting that the more concentrated/viscous solution prevented higher degrees of cross-linking and that more waste products were generated. To investigate the optimum degree of cross-linking achievable with a 5:1 molar ratio, the reaction was left running overnight (γ CPCD-2) but found that no hydrogel was formed even after 22 h at 30°C , and the PEG: γ C D unit ratio was 3.7:1 (Table 1). This is a considerable advantage for process scale-up because there is no need to monitor the reaction to produce the cross-linked material with desired properties.

Thermal analysis by DSC and TGA revealed the glass transition (T_g), melting (T_m), and charring (T_c) temperatures of the

new materials (Table 1 and Figures S3 and S4) and showed how the degree of cross-linking affected these properties. The thermal properties of the γ CPCD samples were midway between those of the precursors PEGDGE and γ CD (SI, Figure S5).^[26] The glass transition temperature was affected by the morphology of the material and was inversely proportional to the degree of cross-linking, which translated into the extra free volume of the sample due to the presence of the flexible PEG units.^[8] In amorphous γ CPCD samples, the PEG chains maintained their movements and reduced the solid–solid transition (vitrification) point of the cross-linked polymer. Another effect that could explain the differences in T_g among the various samples is the number of PEG entanglements: a higher number of PEG chains with one free side could improve the mobility of the entire sample. This effect might explain the lower T_g of sample γ CPCD-5 (Table 1), which was prepared under the same reaction conditions as γ CPCD-1 to determine the reproducibility of the process. Also, some PEG units may have cross-linked with each other, resulting in a more random distribution of cross-links between each γ CD unit and thus offering further opportunities for mobility among the soft parts of the polymer. The melting (T_m) and charring (T_c) temperatures of samples γ CPCD-1, γ CPCD-2, γ CPCD-3 and γ CPCD-5 were similar, the two transitions can be considered as unique phenomenon, but were slightly higher for sample γ CPCD-4 (Table 1). This may reflect the higher number of PEG entanglements in the molecule or different links among the flexible PEG units. TGA showed the same mass loss evolution for all γ CPCD samples (SI, Figure S4). A small loss of mass (<5%) at approximately 80 °C was associated with the evaporation of residual water in the molecules indicating that the samples inherited the hygroscopic properties of γ CD. The two other steps were associated with glucose unit's charring/decomposition at ~255 °C and the decomposition of PEG units at ~340 °C. The amorphous surface of a γ CPCD-1 sample was compared to the microcrystalline form of γ CD by SEM (Figure 5). The high content of flexible PEG chains was confirmed by the surface of γ CPCD-1 at 50 $\times$ magnification and the amorphous polymeric nature of the material.

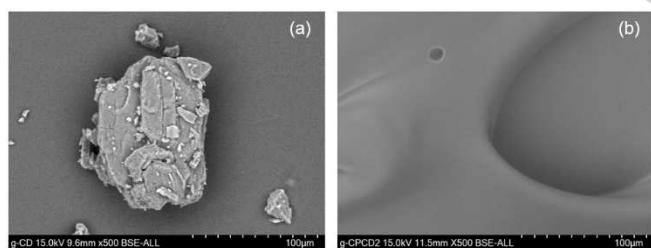


Figure 5. Scanning electron micrographs of (a) crystalline γ CD and (b) amorphous γ CPCD-1 from PEGDGE: γ CD molar ratio of 5:1.

To investigate the possibility of introducing additional cross-links into the soluble γ CPCD polymers, thus producing an insoluble polymeric material with an even lower T_g and better processability, curing was carried out by mixing sample γ CPCD-1 with the same PEGDGE cross-linker used in the cross-linking reaction. The mixture was placed in an oven at 70 °C for 8 days in the presence of a scandium curing catalyst to accelerate the formation of C–O–C bonds between the γ CPCD and PEG units. ^1H NMR spectra (SI, Figure S6) of soluble fractions in $\text{DMSO}-d_6$ recorded at regular intervals during the curing process revealed that all the PEGDGE cross-linker was consumed. During curing,

the sticky, white and mouldable γ CPCD gradually changed into a transparent, rubbery material that was no longer mouldable. This reflected the formation of a 3D cured γ CPCD (*Cur*- γ CPCD) structure that was insoluble in distilled water and common organic solvents. However, the cured sample swelled in distilled water (by 200%), chloroform (by 133%), methanol (by 65%) and ethanol (by 29%) for example (SI, Table S2) suggesting that the new γ CD-based polymer was a good candidate for medical applications (such as the preparation of controlled-release formulations). The T_g of the *Cur*- γ CPCD was –37 °C, significantly lower than its precursor (–19 °C), demonstrating that the insertion of additional flexible PEG chains helped to retain the favourable mechanical properties of the binder at very low temperatures.

Finally, to investigate the effect of further chemical modification on the properties of the soluble cross-linked material, sample γ CPCD-1 was converted into its nitrated derivatives γ NCPCD using concentrated nitric acid. The functionalisation of samples γ NCPCD, was confirmed by ^1H NMR in acetone- d_6 (SI, Figure S7). The broad signals revealed that the polymeric structure had not broken down during nitration reaction. Compared to γ CPCD, new signals were observed in γ NCPCD derivative with downscale shifts confirming that nitration had taken place. The shifts were due to the presence of electronegative environments neighbouring the γ CD and PEG units following the addition of nitrate groups ($-\text{ONO}_2$) to the polymeric structure. DSC showed that the T_g of γ NCPCD had increased to positive temperatures. The average T_g of +19 °C was higher than that of the γ CPCD by ~38 °C. A similar trend in T_g values were also observed after the nitration of β CPCD.^[27] The nitration of γ CPCD was also highlighted by the decomposition energy of 1525–1915 J g^{-1} at the peak temperature of 190 °C (SI, Figure S8).

In summary, a set of cross-linked γ CDs was synthesised from the cross-linking reaction of crystalline γ CD with non-toxic PEGDGE in aqueous alkaline solution. All γ CPCD cross-linked samples were soluble in water and various organic solvents and exhibited amorphous material properties with T_g values as low as –23 °C. Furthermore, the cross-linked product (γ CPCD) was modified by i) curing reaction using additional PEGDGE cross-linker to form an insoluble polymer (*Cur*- γ CPCD) and ii) nitration reaction to give nitrated derivative (γ NCPCD) soluble in organic solvents. The cured polymer displayed excellent swelling properties in various solvents (up to 200% in distilled water) and an even lower T_g of –37 °C while the nitrated derivative showed good thermal stability up to 190 °C and T_g at +19 °C. This study highlighted that γ CPCD in its various forms could be developed as a polymeric material in food and pharmaceutical formulations, with the swelling properties of *Cur*- γ CPCD making it particularly interesting as a controlled drug delivery system and the nitrated γ NCPCD as active pharmaceutical ingredient. The ability of these systems to solubilise and stabilise a variety of molecules is under investigation foreseeing a synergy of γ CD toroid and network morphology.

Supporting Information Summary

Materials and methods, Synthesis of cross-linked γ CD (γ CPCD), Curing of cross-linked γ CD (*Cur*- γ CPCD) in the oven, Synthesis of nitrated cross-linked γ CD (γ NCPCD), ^1H NMR spectra, FTIR spectra, DSC/TGA data and Swelling Studies are available online as Supporting information (SI).

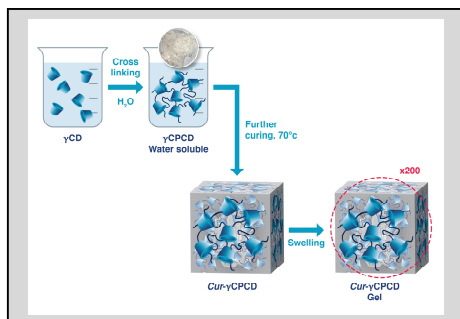
Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. This study was part of an MSc thesis project carried out by Miss Morgan Bolton whose MSc was funded through UK MoD. We would like to thank Mr Matthew Hodgson, Graphic and Media Designer at Cranfield Defence and Security Learning Services, for his contribution with the graphical abstract.

Keywords: cross-linked γ -cyclodextrin; water soluble; non-toxic cross-linker; glass transition; swelling

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Entry for the Table of Contents



The properties of γ -cyclodextrin (γ -CD) have been modified by cross-linking reaction in water. The cross-linked product (γ CPCD) containing polyethylene glycol units (P) was soluble in water. Its properties were further modified by additional curing with the same cross-linker (P) at 70 °C. The cured derivative showed excellent gel characteristics with a swelling power up to 200%.



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2021-08-16

Wiley

<https://doi.org/10.1002/slct.202101963>

Dossi E, Bolton M, Kister G, Afsar A. (2021) Cross-linking of α -cyclodextrin using non-toxic polyethylene glycol spacer units. ChemistrySelect, Volume 6, Issue 3, August 2021, pp. 7727-7731