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## Liquid Chromatography at Critical Conditions (LCCC): Capabilities and Limitations for Polymer Analysis

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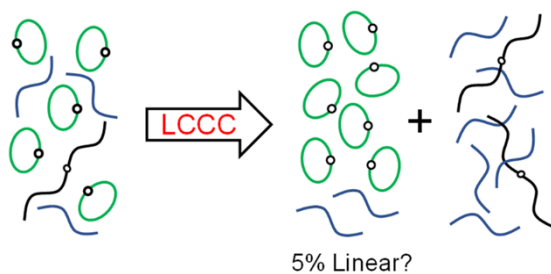
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### Abstract

This paper investigates liquid chromatography at critical condition (LCCC) for polymer analysis. Based on controversial claims on the separation of cyclic polymers from linear analogues in the literature, the efficiency of LCCC for separation and purity analysis is questioned. Polyisobutylene (PIB) and poly(3,6-dioxo-1,8-octanedithiols) (polyDODT) were used for the study. The structure of low molecular weight cyclic and linear polyDODT was demonstrated by MALDI-ToF. NMR did not show the presence of thiol end groups in higher molecular weight PIB-disulfide and polyDODT samples, so they were considered cyclic polymers. When a low molecular weight polyDODT oligomer with only traces of cycles, as demonstrated by MALDI-ToF, was mixed with an  $M_n = 27K$  g/mol cyclic sample, LCCC did not detect the presence of linear oligomers at 6 wt%. Based on the data presented here, it can be concluded that the LCCC method is not capable of measuring  $< 6$  wt% linear contamination so earlier claims for cyclic polystyrene (PS) samples purified by LCCC having  $< 3$  % linear contaminants are questioned.

TOC

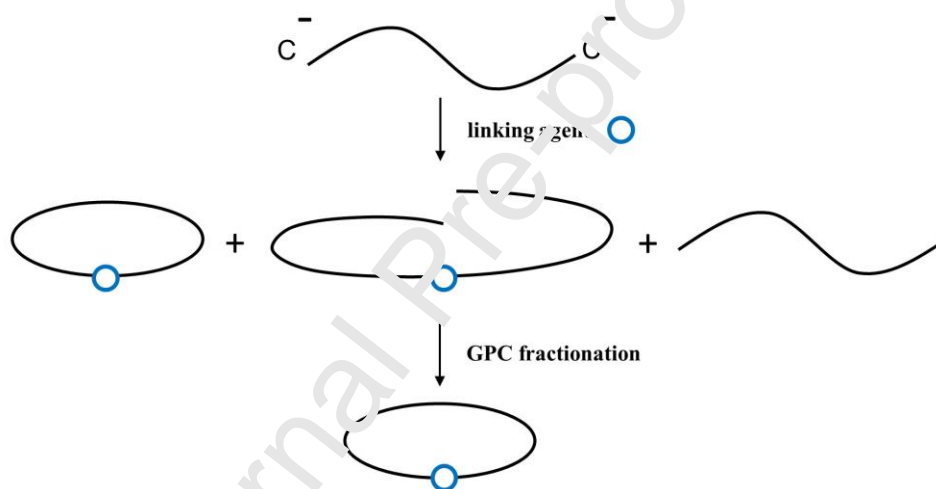
Liquid Chromatography at Critical Condition



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## 1 Introduction

Despite progress in the synthesis of polymer macrocycles, which has created materials of great fundamental interest, limitations in sample size and/or purity remain obstacles to reconciling controversial results and leave the dynamics of cyclic polymers shrouded in uncertainty. Most rheological studies examine cyclic polymers made by linking the two ends of linear chains made by anionic living polymerization under very dilute conditions, inherently leaving linear chains in the final product (Figure 1).



*Figure 1 Synthesis of cycles by connecting the two ends of linear chains made by anionic polymerization.*

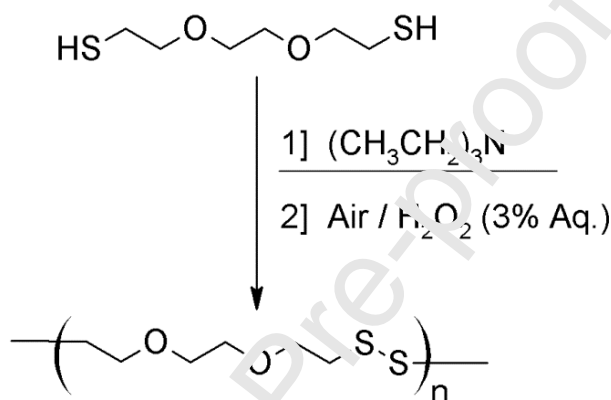
Several linking chemistries have been investigated [1–10], but in all cases a mixture of linear (single terminated, coupled double or higher condensation degrees) and cyclic polymers are obtained. It was also shown that the cyclic content decreases with increasing molecular weight [2], leading to difficulties in fractionating the final products to yield pure cyclic polymer. Contradictory observations regarding cycle dynamics are often attributed to linear contaminants,

fueling ongoing controversy [4,8,10–12]. Since the end-linking methods are not quantitative, the cycles must be separated from linear chains. Early efforts by fractionation by preparative Size Exclusion Chromatography (SEC) have been questioned [8]. The advent of Liquid Chromatography at the Critical Condition (LCCC) led to optimism that a better separation of linear chains from the cycles can be achieved, though the results have been less than fully satisfactory [8,12–16]. The critical condition is usually defined as the point at which the weak attractive enthalpic interaction effect is exactly compensated by the entropic exclusion effect for a polymer. In other words, LCCC operates at the transition of size exclusion and adsorption modes of liquid chromatography, so in principle polymers with various functional groups and architectures (e.g., cyclic and linear polymers) can be separated, regardless of size. However, the initial optimism may be misplaced. To our knowledge, all LCCC studies of cyclic systems have investigated the separation of cyclic polystyrene (PS) from linear analogues in a mixture formed by the ring closure procedure (see Figure 1). Even for a fixed backbone structure, reproducibility across laboratories remains elusive. For example, drastically different rheological properties were reported for similar molecular weight cyclic PS, using similar LCCC procedures for purification [7,10]. Lee *et al.* demonstrated the extreme sensitivity of the critical condition to temperature, solvent composition and column pore size [6], but most publications do not specify the temperature used. Similarly extreme sensitivity is reported for a subtle change in backbone structure: deuteration of 120 kg/mol cyclic PS dramatically decreased the elution time from 6 minutes to 3.7 minutes [7]. Most groups have reported the use of hydrophobized (C18) silica gel column (Hypersil or Eurogel) with 100 Å pore size, and solvent-non-solvent mixtures ( $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN}$  or THF/Hexane) [5,7]. Most work has reported that high molecular weight cyclic PS eluted after the linear counterparts. However, Lee *et al.* reported that when they used a

Hypersil/100 Å and CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>CN 57v/43v solvent mixture, a small cyclic PS with M<sub>w</sub> = 6,500 g/mol eluted earlier than the linears [6]. Cho *et al.* showed that the elution time of cyclic samples increased with increasing molecular weight, but the smallest cyclic PS with M<sub>w</sub> = 5000 g/mol barely separated from its linear counterpart [7]. Pasch *et al.* found a similar problem for smaller cyclic PS: linear PS precursors and cycles of M<sub>w</sub> = 3300 and 8600 g/mol both eluted at similar times in THF-hexane 34.6/65.4% mixture, limiting effective separation [5]. He showed that good separation could be obtained when he used a bare silica column. He also demonstrated the effect of end groups on linear chains and dangling groups on the cycles by comparing RI and UV traces. Thus the literature on LCCC to separate systems as close as linear and cyclic counterparts remains controversial, unlike in the case of block copolymers where LCCC has proven to be highly productive [17,18].

Based on the above examples, we second Lee's suggestion [19] that the differences found by different groups may be related to the different coupling chemistries used for closure of the cycles. McKenna *et al.* compared cyclic PS prepared by two different linking chemistries [4]. In retrospect, the differences they found in rheological behavior could be attributed to the different chemistries leading to different levels of linear contamination. Doi's rheological studies used yet another synthetic method [12,20], while Kapnistos used Roovers' original samples, but now fractionated by LCCC [8]. Pasch used a different, multistep synthesis that resulted in cycles containing various side groups [5]. Pasquino mentions using anionic methods with LCCC purification with no further details [16]. Thus we contend that LCCC may not yield cyclic polymers with the purity claimed (<1-3 wt% linear contaminants). Therefore, we set out to investigate LCCC with a different polymer family that was shown to produce inherently very pure cycles under selected conditions without any linear contaminants. This new family is

disulfide polymers made by Redox Radical Recombination Polymerization (R3P) of dithiols. This “green” process, using recyclable triethylamine (TEA) aqueous hydrogen peroxide and air, was invented in the Puskas lab and used to polymerize poly(3,6-dioxa-1,8-octanedithiol) (see Scheme 1) containing only disulfide links, making the products fully biodegradable to monomer [21–24].



*Scheme 1 R<sup>3</sup>P producing polyDODT.*

Under strongly oxidizing conditions, cyclization is preferred, yielding high molecular weight cyclic polyDODT with no “foreign” groups. MALDI-ToF of the low molecular weight fractions of a poly(DODT) with  $M_n = 164\text{K g/mol}$  showed pure cyclic polymer (Figure 2A and B). The structure of the polymers somewhat resembles poly(ethylene oxide), but it is an amorphous, non-crystallizing, rubbery polymer at room temperature, with  $T_g$  around  $-50\text{ }^\circ\text{C}$ . Weaker oxidizing conditions may result in a mixture of cyclic and linear macrodithiols. In fact, so far, we have not been able to make pure linear polyDODT of high molecular weight. However, when less than two equivalents of 3wt%  $\text{H}_2\text{O}_2$  was used, low molecular weight oligomers ( $M_n \sim 1000\text{-}2000$  g/mol) containing mostly linear chains with traces of cycles formed, as demonstrated by

MALDI-ToF (2C) [25]. The enlarged spectrum in 2D shows equal intensities with  $m/z = 1105.247$ , 1285, 284 and 1465.318 for the sodiated hexa-, hepta- and octamer with thiol end groups. Very small signals can also be seen for cyclic structures at 2  $m/z$  units lower, marked with an arrow in Figure 2D as "cyclic" that could not be seen in the NMR. The other two smaller distributions were assigned to chains capped with one  $m/z = 1175.269$ ) or two ( $m/z = 1245.495$ )  $\text{SO}_3\text{Na}$  groups, possibly formed by oxidation [26].



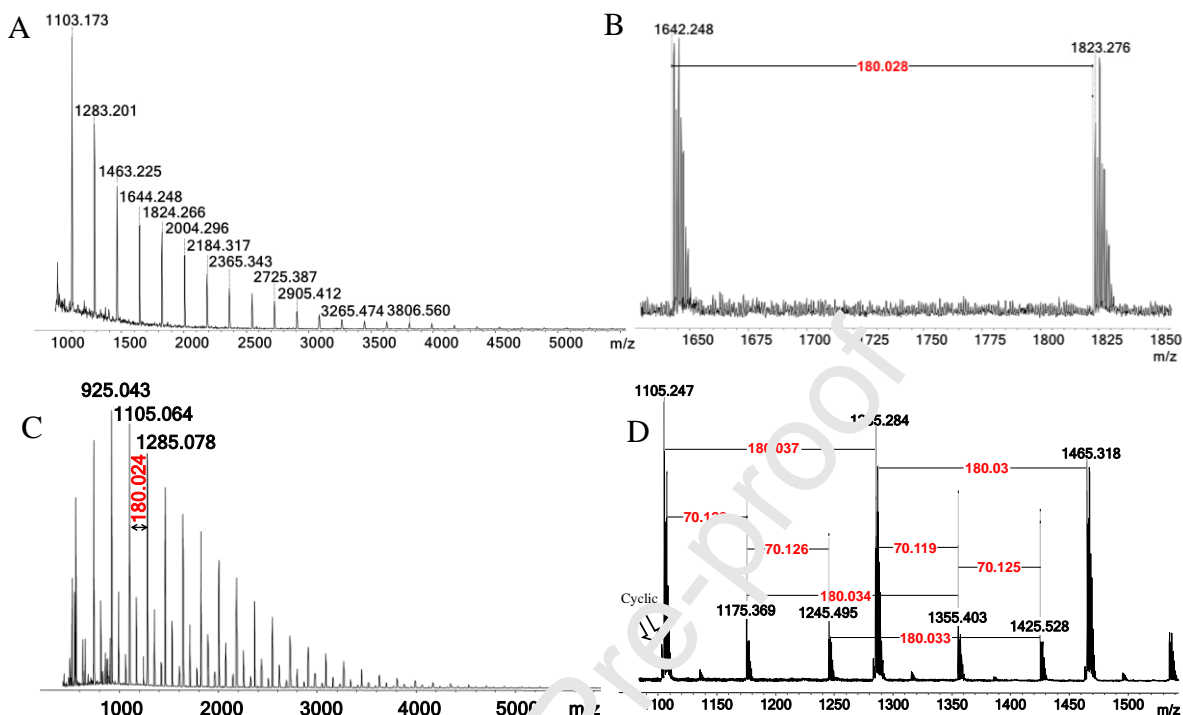
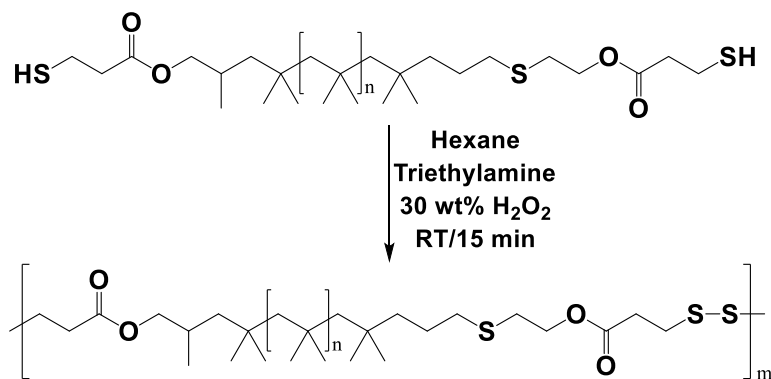


Figure 2. MALDI-TOF spectra of polyDCDTs: (A) cyclic, full region and one step change enlarged, where the 180.028 m/z change represents one repeat unit (B); linear, full region (C) and or one step change enlarged (D).

We also made cyclic polyisobutylene (PIB)-disulfide using 30 wt% H<sub>2</sub>O<sub>2</sub>, for which 750 MHz <sup>1</sup>H-<sup>13</sup>C correlation NMR did not reveal any thiol end groups [27]. Together with the additional evidence (Raman spectroscopy, SEC conformation analysis and higher T<sub>g</sub> than the linear counterpart), they can be considered cyclic polymers. However, in this case the repeat units are macromonomers containing “foreign” ester groups as shown in Scheme 2:



*Scheme 2 Synthesis of cyclic PIB-disulfide by R3P.*

In this paper we discuss LCCC of PIBs and polyDODTs and the results will highlight the limitations of this method as well as setting upper bounds on the detection limit of linear content of these materials.

## 2 Materials and methods

### 2.1 Materials

3,6-dioxa-1,8-octanedithiol (DODT) 95% (Aldrich), Triethylamine (TEA) (TCI), H<sub>2</sub>O<sub>2</sub> 30 wt% (Sigma-Aldrich), Acetone technical grade (Sigma), tetrahydrofuran (THF) HPLC grade non-stabilized (Fischer Scientific), methanol HPLC grade (Fischer Scientific). For mass spectrometry THF (anhydrous, 99.9%, Aldrich), 1,8,9-anthracenetriol (dithranol, >97%; Alfa Aesar), sodium trifluoroacetate (>98%; Aldrich) or silver trifluoroacetate (98%, Aldrich). Polyisobutylene standards  $M_n = 2200, 30,700$  and  $118,200$  g/mol ( $\bar{D} = 1.30, 1.26, 1.50$ ) (Polymer Standards Co.). Functionalized PIBs were synthesized as reported earlier and are summarized in Table 1 [27,28]. The cyclic PIB disulfide (PIB-SS) was synthesized as reported earlier ( $M_n = 38,000$  g/mol,  $\bar{D} = 1.60$ ) [27].

Table 1 Functionalized PIBs

Structure and $M_n$ (g/mol)	$\bar{D}$
OH-PIB-OH 1200	1.30
OH-PIB-OH 1800	1.30
OH-PIB-Allyl 1450	1.36
HS-PIB-SH 1200	1.30

## 2.2 Synthesis of polyDODT

DODT monomer and TEA were mixed first for 20 minutes in an open 3 neck round bottom flask. In the case of experiments 1 and 3 (Table 2) 30 wt% aqueous  $H_2O_2$  was added to the DODT-TEA mixture in 10–15 aliquots of equal volume during a time period of 60 and 90 minutes to keep the reaction temperature relatively constant. Air was bubbled into the reaction flask for 5 minutes, followed by stirring using a magnetic stir bar in an open environment for 2 hours. The precipitated polymer was removed from the reaction flask. In the case of experiments 2 and 4 (Table 2), the mixture was added at once to 3 wt% aqueous hydrogen peroxide ( $H_2O_2$ ), allowed to react for 1 more minute and poured into excess methanol to precipitate the polymer. The polymers were washed first with water then with acetone for 4 x 30 minutes to remove residual TEA and monomer. The excess acetone was decanted, and the polymers were dried in a vacuum oven until a constant weight was achieved.

Table 2. Synthesis of polyDODTs.

Sample ID	DODT (mmol)	DODT:TEA: $H_2O_2$ molar equivalency
<b>1</b>	110.58*	1 : 3 : 2
<b>2</b>	3.21**	1 : 2.3 : 2
<b>3</b>	27.10*	1 : 3 : 2
<b>4</b>	3.22**	1 : 2.3 : 2

\*30 and \*\*3 wt% aqueous solution of  $H_2O_2$ .

### 2.3 Size Exclusion Chromatography (SEC)

The SEC instrument consisted of an Agilent 1260 infinity isocratic pump, a Wyatt Eclipse DUALTEC separation system, an Agilent 1260 infinity variable wavelength detector (UV set at 254 nm), a Wyatt OPTILAB T-rEX interferometric refractometer, a Wyatt DAWN HELOS-II 18-angle static light scattering detector (MALS) with a built-in dynamic light scattering (DLS) module, an Agilent 1260 infinity standard autosampler. 6 StyragelVR columns (HR6, HR5, HR4, HR3, HR1, and H0.5) were thermostated at 35°C. THF was the mobile phase at a flow rate of 1 mL/min continuously distilled from CaH<sub>2</sub>. In each case 100  $\mu$ L, 2 mg/mL polymer solution was injected. The results were analyzed using the ASTRA 5 software (Wyatt Technology), using  $dn/dc = 0.108$  and  $0.132$  for PIB [29] and polyDOLT [24], respectively. UV absorption coefficients were obtained assuming 100% mass recovery.

### 2.4 Proton Nuclear Magnetic Resonance spectroscopy (<sup>1</sup>H-NMR)

NMR spectra were recorded on Varian NMR 500 and 750 MHz instruments using deuterated chloroform (Chemical Isotope Laboratories, 99.8% CDCl<sub>3</sub>). The resonance of non-deuterated chloroform at  $\delta=7.27$  ppm for <sup>1</sup>H and  $\delta=77.23$  for <sup>13</sup>C ppm was used as internal reference.

### 2.5 Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-ToF)

MALDI-ToF mass spectra were acquired with a Bruker UltraFlex-III time-of-flight (ToF) mass spectrometer (Bruker Daltonics, Billerica, MA) equipped with a Nd:YAG laser (355 nm), a two-stage gridless reflector, and a single stage pulsed ion extraction source. Separate THF (anhydrous, 99.9%, Aldrich) solutions of polymer (10 mg/mL), 1,8,9-anthracenetriol (dithranol,

20 mg/mL, >97%; Alfa Aesar), sodium trifluoroacetate (10 mg/mL, >98%; Aldrich) or silver trifluoroacetate (10 mg/mL, 98%, Aldrich) were mixed in a ratio of 10:1:2 or 14:1:4 (matrix:cationizing salt:polymer), and 0.5  $\mu$ L of the resulting mixture was introduced on to the MALDI target plate and allowed to dry. The spectra were obtained in the reflectron mode. The attenuation of the nitrogen laser was adjusted to minimize unwanted polymer fragmentation and to maximize the sensitivity. The calibration of mass scale was carried out externally using poly(methyl methacrylate) or polystyrene standards having similar molecular weight as the sample.

### 2.6 Liquid Chromatography at Critical Conditions (LCCC)

The set-up consisted of a Macherey-Nagel Nucleosil® C18 column (100 Å pore size, 5  $\mu$ m particle size, length 250 mm, inner diameter 4.6 mm) operated at 35°C and a flow rate of 0.5 mL/min in every case. Eluents were mixed from HPLC grade methanol and THF. Polymer solutions were prepared using the eluent mixtures with 0.05 wt% polymer concentration. The results were analyzed using the ASTRA 7 software (Wyatt Technology).

## 3 Results and Discussion

### 3.1 PIBs

*Banerjee et al.* established LCCC condition for PIBs with  $M_n < 3000$  g/mol with 19.5 wt% MeOH in THF solvent mixture at 35°C column temperature [30]. The same conditions were used in this study. Figure 3 shows the differential Refractive Index (dRI) LCCC traces of HO-PIB-OH, HO-PIB-Allyl, HS-PIB-SH synthesized by our group [28,31] and a PIB standard (2.2K g/mol from Polymer Standards). For this latter, the end groups are not specified, but likely are a  $\text{CH}_3$  - at one end and a double bond at the other. It is clear that the linear PIB oligomers separate based on their end groups (although the retention time of HS-PIB-SH is very close to those of the diols) rather than eluting together based on their linear topology; when end groups are identical,

linear chains of different length do elute together: two HO-PIB-OH samples of different  $M_n$  had the same retention time at 6.26 minutes in 19.5 wt% MeOH in THF, close to that reported for a linear HO-PIB-OH with a different structure by *Banerjee et al* (see Tables

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Table 3 and Table 4) [30].

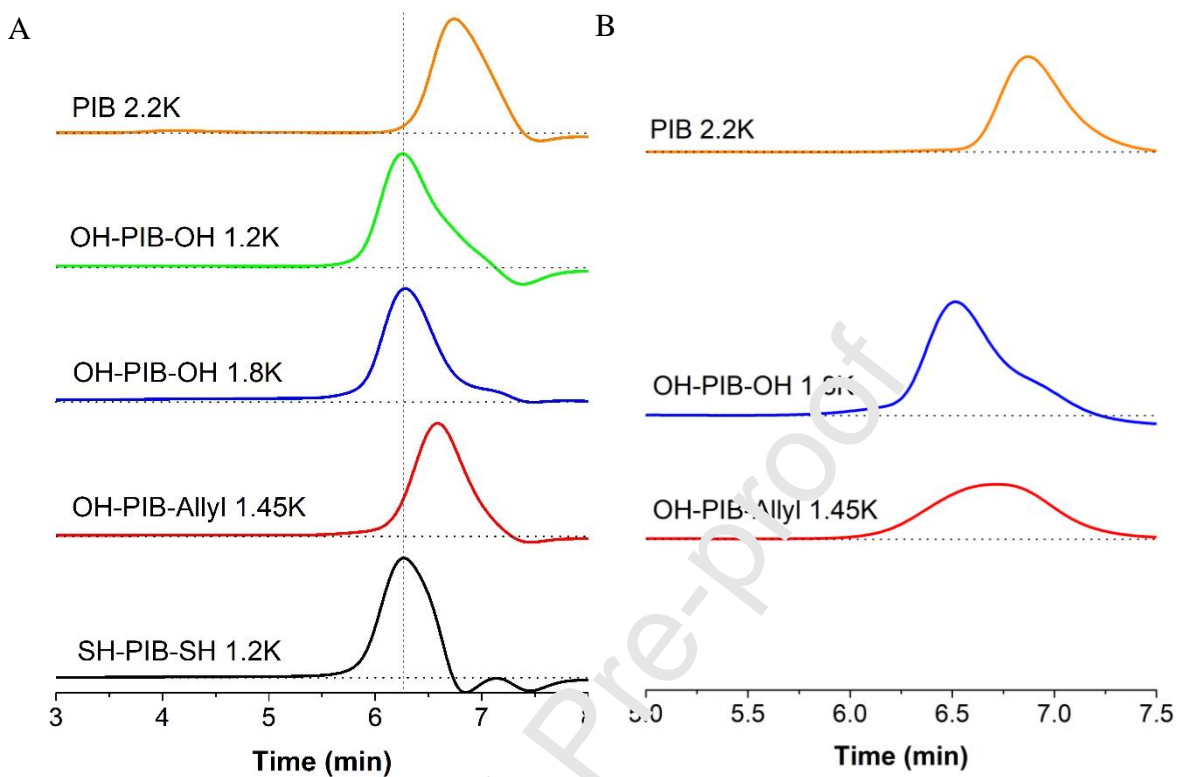


Figure 3 LCCC chromatograms of PIBs with different end-groups: (A) dRI traces, (B) UV traces vertical line: 6.26 minutes.

Table 3 Retention times (in minutes) of PIBs in 19.5 wt% and 10wt% MeOH/THF

Structure and $M_n$ (g/mol)	dRI (min)	
	wt% MeOH	
	19.5	10.0
PIB Standard L-2.2K	6.74	6.17
OH-PIB-OH 1200	6.26	5.89
OH-PIB-OH 1800	6.27	5.96
OH-PIB-Allyl 1450	6.58	6.19
HS-PIB-SH 1200	6.27	5.85
PIB Standard L-31K	-	4.38
PIB Standard L-118K	-	4.06
PIB-SS (cyclic, C-38K)	-	4.20

Table 4 Retention times from the work of Banerjee et al. of PIBs with different end groups and  $M_n \sim 2000$  g/mol in 19.5 wt% MeOH/THF[30]

Structure (g/mol)	dRI (min)
PIB-diol	5.8
PIB-monool	5.9
PIB-Cl	6.4
PIB diallyl	6.5
PIB-diolefin	6.5

While PIB is transparent in UV, the purchased PIB2.2K standard displayed a very strong UV trace (Figure 3B). This must be due to a UV-active functional groups – likely a double bond. For comparison, Figure 3B also displays UV traces for OH-PIB-Allyl and HO-PIB-OH..



Table 3 also lists retention times in 10% MeOH in THF, because higher molecular weight PIBs did not dissolve in the 19.5 wt% MeOH/THF mixture. The retention times of the low molecular weight PIBs were shorter in 10 wt% MeOH/THF but not much. The reason for the very small difference in retention times between 19.5 and 10% MeOH is not clear. However, the higher molecular weight PIB standards (L-31K and L-118K) eluted very close to each other (despite having very different molecular weights) as expected for the critical condition. Figure 4 presents the LCCC traces. Figure 4A also shows the UV signal of the samples. Since PIB is transparent in UV, any signal represents the presence of a UV-active residue in the chain.

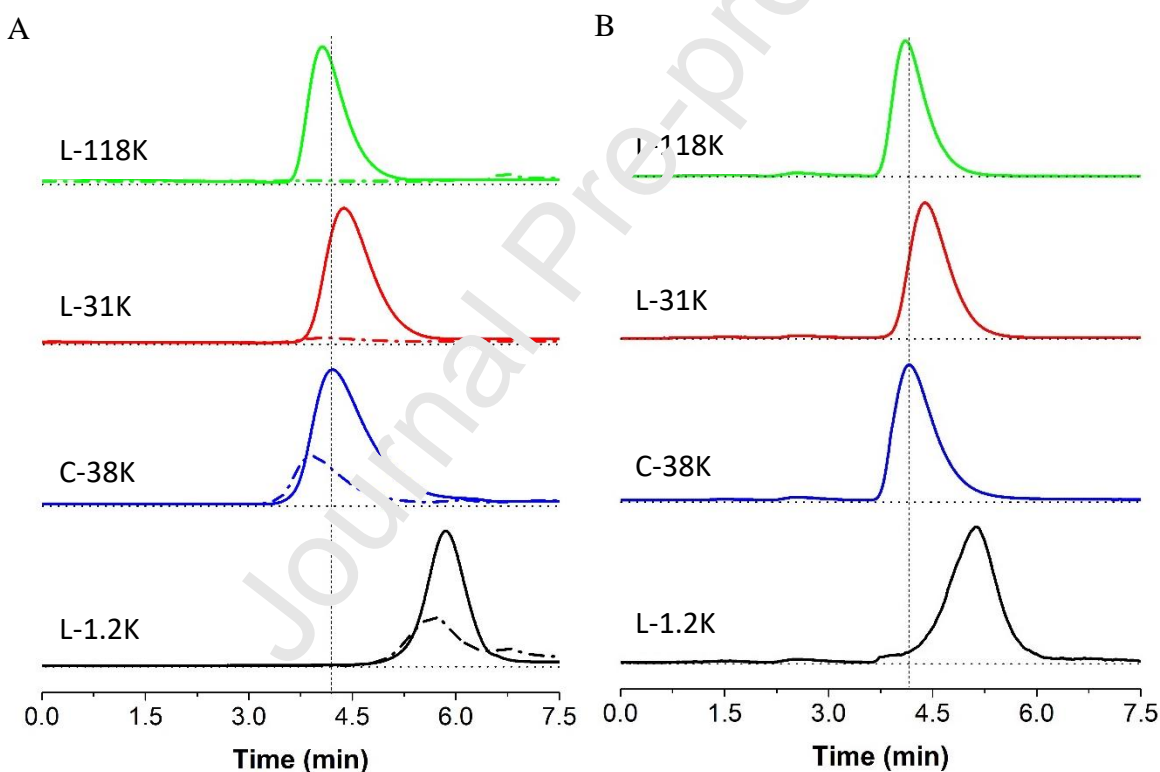


Figure 4. LCCC chromatograms of PIB standards, a cyclic poly(PIB-disulfide) and its HS-PIB-SH precursor: (A) dRI (solid line) and UV (dash-dot line) traces and (B) LS traces. L stands for linear and C stands for cyclic polymers, and the numbers represent  $M_n$  values. Vertical line: 4.20 minutes.

The UV signal of the 38k cyclic poly(PIB-SS) is shifted toward the higher molecular weight L-31K and L-118K standards, relative to the HS-PIB-SH macromonomer. The L-31K and L-118K PIB standards do not show any UV signals (dash-dot in Figure 4A) because either they do not have UV-active end groups or, if they have an olefin end group the sensitivity of the detector is not enough to show a signal. Figure 4 also shows that the L-118K standard elutes first, while the cyclic poly(PIB-SS) (C-38K) elutes a bit faster than the L-31K PIB standard. We postulate that the cyclic poly(PIB-SS) containing polar ester groups interacts with the non-polar column less than the purchased linear standards whose end groups are unknown. Due to their high molecular weight the end groups are expected to have little influence on the UV absorption – accordingly, the two linear PIB standards show no UV absorption. The cyclic poly(PIB-SS), on the other hand, shows a distinct UV trace. The area under the UV trace relative to the dRI trace is 34.5% for the cyclic poly(PIB-SS) (C-38K), very close to 39.8% calculated for the HS-PIB-SH 1200 (L-1.2K). This is indirect evidence for the structure of the repeat units in the C-38K.

In summary, under the conditions investigated the cyclic poly(PIB-SS) (C-38K) eluted before the PIB L-31K linear standard possibly due to less interaction of the cycle with the column. However, the small difference in the retention times would make separation of cyclic poly(PIB-SS) from linear PIB impossible. We plan to use a bare silica column to get better separation, similarly to Pasch's approach.

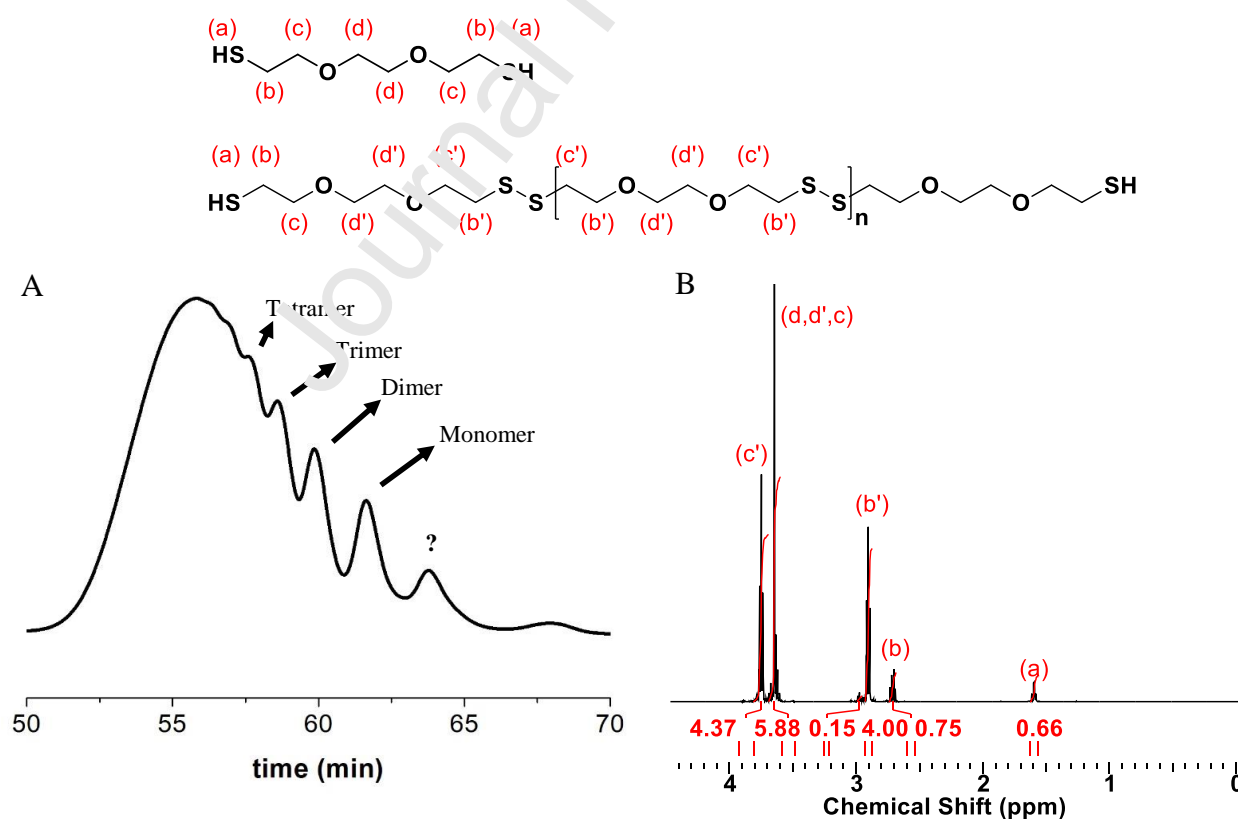
### 3.2 *PolyDODT*

Five polyDODT samples were investigated and the SEC data are summarized in Table 5, together with the experiment numbers in Table 2.

Table 5. Molecular weight data of polyDODT samples.

Sample ID	Yield (%)	$M_n$ (g/mol)	$M_w$ (g/mol)	$\bar{D}$
1: C-370K	90	366,700	556,000	1.5
2: C-78K	89	77,800	140,000	1.8
3: C-75K	92	75,300	103,000	1.4
4: C-27K	91	27,400	52,000	1.9
5 L-1.3K	85	1,300	2,100	1.4

The fifth sample in Table 5 is the linear oligomer whose MALDI-ToF is shown in Figure 2C and D. Figure 5A shows the UV SEC traces. The oligomer peaks up to tetramers, assigned based on the retention time-peak molecular weight constructed for polyDODT, are well resolved. SEC gave the number average molecular weight of  $M_n = 1300$  g/mol with  $\bar{D} = 1.4$ . Figure 5B also shows the  $^1\text{H}$  NMR spectrum with peak assignment, in comparison with the monomer. The assignments correspond to a linear oligomer with thiol end groups.

Figure 5 (A) SEC UV trace and (B)  $^1\text{H}$ -NMR spectrum of 1.3K linear polyDODT sample.

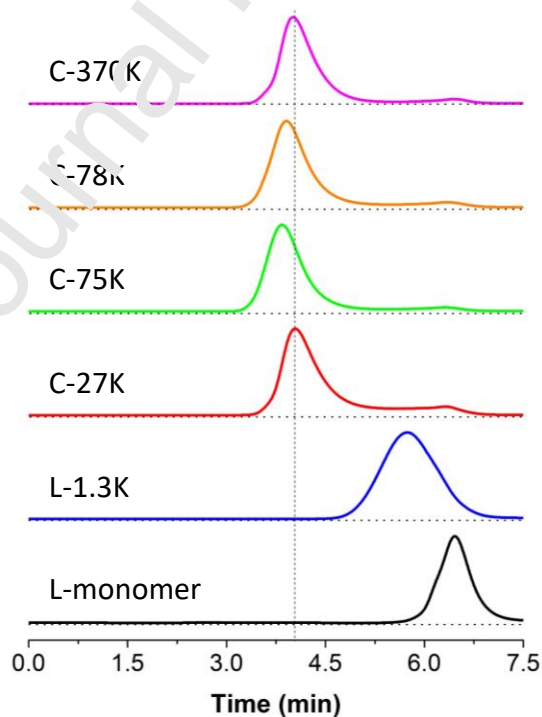
The signal of the thiol proton from the monomer (a) practically did not change (1.58 vs 1.60 ppm). The signals b and c ( 2.68 and 3.61 ppm) shifted from their position from the monomer to b' and c' ( 2.98 and 3.75 ppm) in the polymer on account of the disulfide bond formation. The position of the internal proton signals in the monomer (d 3.62 ppm) and the polymer (d' 3.65 ppm) are very close and overlap with the polymer end group protons (c). The signal at 2.68 ppm is assigned to protons (b) in the polymer end groups. In cyclic polymers only b', c' and d can be seen (not shown).

LCCC involves the use of solvent mixtures of at least two components, with a “good” and a “bad” solvent of the polymer. Very little data are available for polyDODT so the  $\delta$  solubility parameter was calculated [32].  $\delta = 11.0 \text{ cal}^{1/2} \text{ cm}^{-3/2}$  was obtained for polyDODT, close to PS at  $10.6 \text{ cal}^{1/2} \text{ cm}^{-3/2}$  and farther from PEO and PIB at  $9.4$  and  $7.7 \text{ cal}^{1/2} \text{ cm}^{-3/2}$ . In light of this and that polyDODT dissolves easily in THF for the LCCC experiments an THF/MeOH solution was chosen, similarly to what we used for the PIB. Table 6 shows LCCC retention times based on UV traces for 3 different solvent mixture compositions. There was only a small shift in retention times from 10 wt% to 25 wt% MeOH, but the sample with the highest  $M_n$  dissolved only in the mixture with 10 wt% MeOH at a reduced concentration of 0.16 wt%.

*Table 6. Retention times of polyDODTs in different MeOH/THF compositions.*

MeOH content	10 wt%	19.5 wt%	25 wt%
Sample and $M_n$ (g/mol)	Retention time according to UV peak (min)		
<b>R-370K</b>	4.01	NA	NA
<b>C-78K</b>	3.84	3.93	4.01
<b>C-75K</b>	3.83	3.88	3.93
<b>C-27K</b>	4.04	4.10	4.12
<b>L-1.3K</b>	5.74	-	-
DODT monomer <b>L-0.18K</b>	6.46	6.55	6.56

Figure 6 compares the UV LCCC traces of the polymers and the DODT monomer at 10 wt% MeOH content. The L-1.3K sample shows a single peak at 5.75 minutes. The traces of the first four polymers are also monomodal at around 4 minutes, and are close to each other, regardless of the large difference in  $M_n$  values. The short elution times are also similar to the high molecular weight PIB samples, indicating less interaction with the columns compared to the low molecular weight standards and the monomer (which elutes last at 6.46 minutes) and for which the end groups have a significant influence on the elution times. The 27, 75 and 78K polyDODT samples are cyclic as they did not have thiol end group signals by NMR. The 370K polymer also did not have thiol end group signals but in this case NMR may not be sensitive enough to show end group signals.



*Figure 6. UV LCCC traces of polyDODTs in 10 wt% MeOH/90 wt% THF. L stands for linear and C stands for cyclic polymers, and the numbers represent  $M_n$  values. Vertical line: 4.04 minutes.*

The short retention times of the cyclic samples could be related to the fact that there is likely less interaction with the silica column than in the case of the linear polymer and the monomer having thiol end groups. The polyDODT cycles have no other chemical groups other than the repeat units, shown in Scheme 1. Unfortunately, we could not make linear polyDODTs of high molecular weight, so we can only show that the L-1.3K eluted much later than the cyclic polymers, likely due to more interaction with the column. It is likely that we will find better separation using a bare silica column in the future.

### 3.3 Sensitivity Analysis

The UV molar extinction coefficient calculated from the UV signal of polyDODT ( $\epsilon_{264} = 2.00$  mL/mgcm) was found to be ~30% larger than that of PS ( $\epsilon_{264} = 1.52$  mL/mgcm) thus promising higher sensitivity for the measurement than would be obtained from PS. We calculated the sensitivity of our RI and UV detectors as the 3xnoise (N, baseline fluctuation) to signal (S, peak height) ratio. 200  $\mu$ g of a PS standard ( $M_n = 29,300$  g/mol and  $D = 1.002$ , 100  $\mu$ L with 2 mg/mL concentration) was used, typical for SEC analysis. The RI detector had a 3xN/S ratio of 0.003 and the UV detector had a 3xN/S ratio of 0.0003. Thus the detection limit would be 6  $\mu$ g in the 200  $\mu$ g injected sample, translating to 3 wt%. Thus, the UV detector's detection limit was estimated to be 3wt% linear contamination at best for samples with narrow dispersity. A series of concentrations were made with the linear oligomer of polyDODT (L-1.3K, Figure 2C and D). Based on the UV detector signal, the sample concentration was linearly proportional to both the UV peak heights and UV peak area as demonstrated in Figure 7. At the lowest concentration, the

UV signal was quite weak, which provides an indication of the limit of sensitivity of the setup used in our experiments.

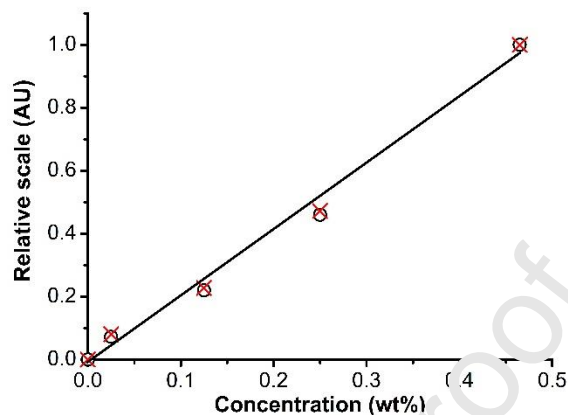


Figure 7. UV-concentration plot of L-1.3K. X – peak area ( $R^2 = 0.9898$ ); o – peak height ( $R^2 = 0.9919$ ).

Next, 6 wt% of the linear polyDODT sample (L-1.3K) was mixed with the cyclic polyDODT sample with  $M_n = 27,400$  g/mol (C-27K). The total polymer concentration was 0.5 wt%, so the concentration of L-1.3K was 0.03 wt%. Five traces are displayed in Figure 8. No signal appears at 5.75 minutes in the mixture. Figure 8 shows the traces of the series with progressively increasing L-1.3K content. The first small signal that can be detected at the position of the mostly linear chain appears when 27 wt% of L-1.3K was mixed with the cyclic sample (0.135 wt% L-1.3K). Integration of the area under the peak assigned to the linear oligomer relative to the 27K cyclic sample gave 28.7% which is in very good agreement with the nominal 27%. The same method yielded 50.3 and 89.1% for the nominal 50 and 93% mixtures.

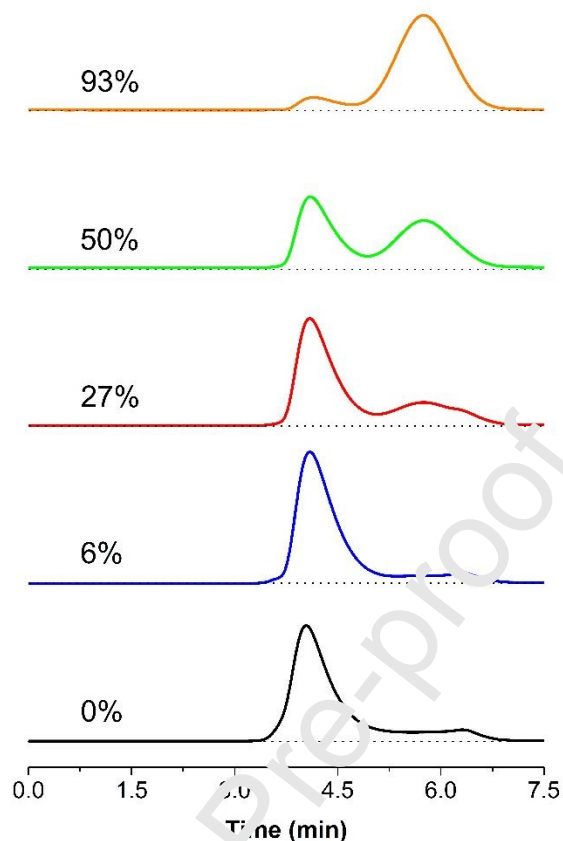


Figure 8. UV traces of L-1.3K – C-27K mixtures with % L-1.3K content in a total of 0.5 wt% mixture, marked on the graphs. 0, 6, 27, 50 and 93% represent 0, 0.03, 0.14, 0.25 and 0.47 wt% absolute L-1.3K concentrations (see also Figure 7.)

Based on the data presented here, it can be concluded that the LCCC method discussed in this work was not capable of measuring  $< 6$  wt% linear contamination.

#### 4 Summary

In conclusion, we found that while LCCC is a nice method to separate polymer samples based on their chemical structure as opposed to their size, but that the molecular weight range might be limited due to solubility differences. Based on our data LCCC under the conditions investigated is not sensitive enough to detect small amounts of linear contaminants in cyclic samples, even in a polymer with higher UV sensitivity (absorption coefficient) than PS. We have provided



evidence that there are caveats that our community must consistently acknowledge when using LCCC to detect or quantify the amount of linear contaminants in a putative cyclic sample. We plan to use a bare silica column for separation in the future, based on Pasch's suggestion [5].

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### Graphical abstract:

### HIGHLIGHTS:

- Liquid chromatography at critical condition (LCCC) – capabilities and limitations
- Investigation of poly(3,6-dioxa-1,8-octanedithiol) (polyDODT) and polyisobutylene-disulfide (PIB-SS)
- Demonstration of linear and cyclic polyDODT structure by NMR and MALDI-ToF
- Demonstration of cyclic PIB-SS by NMR

- Limitations: solubility of low and high molecular weight samples; detection limit of <6 wt%

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