# Normal-phase (Temperature Gradient) Interaction Chromatography – a powerful tool for the characterisation of high molecular weight chain-end functionalised polymers

*Lian R Hutchings*[a], Serena Agostini[a], Matthew E Oti[a] and Jennifer Keth[b]*

[a]Durham Centre for Soft Matter, Department of Chemistry, Durham University, Durham, DH1 3LE, United Kingdom.

[b] Present address: Institute of Organic Chemistry, Johannes Gutenberg University Mainz, Duesbergweg 10-14, 55128 Mainz, Germany

*Corresponding author – l.r.hutchings@durham.ac.uk

**Abstract**

We report here, for the first time, quantitative analysis of end-group functionalization and the extent of end-group modification of polymers with molar mass up to 200,000 $gmol^{-1}$, using a combination of isothermal and temperature gradient interaction chromatography. At such high molecular weights, other common analytical techniques such as MALDI-ToF-MS and NMR spectroscopy are simply unable to offer any quantitative insight into the end-group functionality of polymers. Thus, normal phase isothermal interaction chromatography (NP-IIC) has been used to characterise a series of polystyrene samples, with identical molar mass (c. 90,000 $gmol^{-1}$), each carrying a single chain-end functionality of varying polarity, introduced via a series of end-group transformation reactions - from silyl-protected alcohol → primary alcohol → alkyl bromide. It is shown that the polarity of the functional group

dictates the elution volume of the polymers and that NP-IIC could be used to monitor the quantitative end-group transformation. Thus, rather than estimating the success of the transformation reaction from the very small peaks observed in $^{1}$H-NMR spectra, NP-IIC gives clear chromatographic peaks, whose retention volumes change dramatically only as a consequence of each new chain-end functional group. We also describe the analysis, by a combination of normal phase isothermal and temperature gradient interaction chromatography, of a series of linear polybutadiene samples of varying molar mass (28,000 – 200,000 gmol$^{-1}$) each carrying a single primary alcohol functionality at the chain-end. The primary alcohol functionality enabled the separation of functionalised and non-functionalised polymers, which eluted at different retention volumes despite identical molecular weights. It is all the more remarkable that complete (baseline) resolution can be achieved for two polymers with an identical molar mass (< 200,000 gmol$^{-1}$), which differ only in that one sample is functionalised with a single primary alcohol group.

**Keywords**

Polymer characterization, temperature gradient interaction chromatography (TGIC), anionic polymerization, end-functionalized polymers.

## Introduction

We report here, for the first time, quantitative analysis of end-group functionalization and end-group modification of polymers with molar masses of up to 200,000 gmol$^{-1}$, using a combination of isothermal and temperature gradient interaction chromatography. Other common analytical techniques such as MALDI-ToF-MS and NMR spectroscopy are simply unable to offer any quantitative insight into the end-group functionality of such polymers at such high molecular weights.

Since temperature gradient interaction chromatography (TGIC) was first reported in 1996[1] by Prof. Taihyun Chang, many studies have been carried out on various types of linear

polymers[2-5] in order to compare TGIC characterisation to the more commonly exploited size exclusion chromatography (SEC). However, TGIC came to prominence as a characterisation tool for the analysis of structural heterogeneity of 'model' branched polymers and a significant volume of work has been published in this field[6-15]. It is well understood that SEC separates polymers in terms of hydrodynamic volume (molecular size) rather than molecular weight, although this does not present a particular limitation in the analysis of linear polymers. However, SEC has an inherent drawback, in that it is incapable of separating polymers with identical or nearly identical hydrodynamic volume, which may differ in other molecular parameters such as molecular weight, chain architecture or chain functionality. Such a limitation is a particular concern for the SEC characterization of model branched polymers for structure-property correlation studies. In contrast, TGIC separation, in common with other interaction chromatography techniques, is driven by enthalpic interactions between the solute molecules and the stationary phase and TGIC, in particular, reversed phase (RP) TGIC can exploit these interactions to resolve polymer samples based on molecular weight NOT hydrodynamic volume. Thus RP-TGIC has been used to study structural heterogeneity in star-branched polymers[6-8], H-shaped polymers[9,10], comb-branched polymers[11-13], dendritically branched polymers[14] and the entire field has been recently reviewed[15]. RP-TGIC is characterised by a non-polar, hydrophobic stationary phase, such as $C_{18}$ modified silica, and a mobile phase that is more polar than the stationary phase. Although RP-TGIC is less universal than SEC, it has proved to be a crucially important technique for the characterisation of branched polymers.

Normal phase (NP) TGIC, which exploits a polar stationary phase such as bare silica or diol bonded silica and a less polar mobile phase, has been explored far less as a technique but

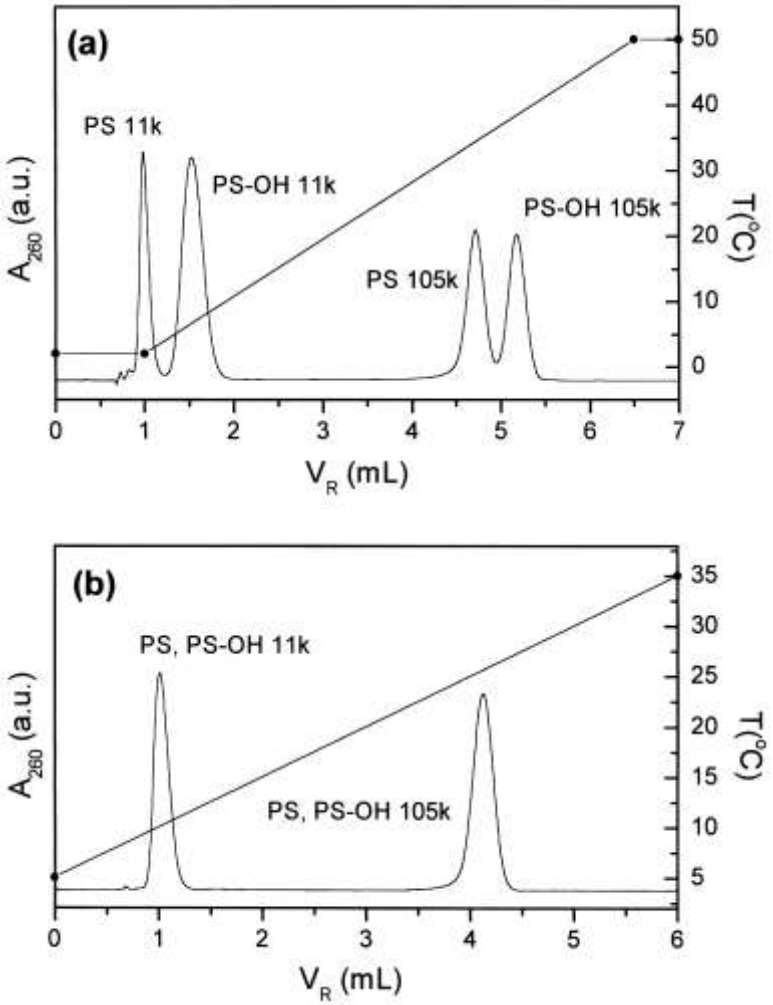



**Figure 1.** TGIC separation of polystyrene samples with different end groups (hydrogen terminated vs. hydroxyl terminated) by (a) NP-TGIC and (b) RP-TGIC[16]. Reprinted from Journal of Chromatography A, volume 910 (1), Lee, W.; Cho, D.; Chun, B. O.; Chang, T.; Ree, M., Characterization of polystyrene and polyisoprene by normal-phase temperature gradient interaction chromatography, pages 51-60, Copyright (2001), with permission from Elsevier.

may prove to be equally valuable in the characterisation of polymers; not in terms of their molecular weight but in terms of functionality. The introduction of chain-end functionality into high molar mass polymers has become a hot topic in the tyre-rubber industry where polar functionalities located at the chain-end can dramatically improve the dispersion of filler particles such as silica and carbon black, but can also lead to improved rolling resistance and fuel efficiency[17]. However, as the molecular weight of the polymer increases, the ability of more common techniques, such as MALDI and NMR, to accurately quantify the degree of chain end-functionalization becomes increasingly challenging. Moreover, since the polymers

used in tyre treads may have a molecular weight of greater than 200,000, even detection of such functional groups becomes a near impossibility. In a single previous report[16] NP-TGIC was shown to be sensitive to the presence of polar end-groups on the polymers and the separation of polymers, not only in terms of molecular weight, but also in terms of functional groups was reported. In this report Chang *et al.* used NP-TGIC for the analysis of hydroxyl chain-end functionalised polystyrene polymers (figure 1). Polystyrene samples with a molar mass of 11,000 $gmol^{-1}$ and 105,000 $gmol^{-1}$ were prepared by living anionic polymerisation. Each sample was divided into two portions and whilst one portion was terminated with methanol, resulting in an unfunctionalised polymer (PS), the other portion was end-capped with ethylene oxide and then terminated to yield polystyrene end-capped with a single hydroxyl group (PS-OH). Chang was able to show that, in a single NP-TGIC experiment, using a mixture of all four samples (PS11K, PS105K, PS-OH11K and PS-OH105K), he was able to resolve the samples both in terms of molecular weight and chain end functionality. When using RP-TGIC, separation was only possible in terms of molecular weight. More recently, and in a similar fashion, Chang reported[18] the use of liquid chromatography at critical conditions (LCCC) and solvent gradient NP-HPLC, to analyse the degree of functionalization of a series of telechelic polyisobutylene diols. In this case, the analysis was also very effective at resolving functional from non-functional polymers although the polymer molar masses were rather modest (less than 5,000 $gmol^{-1}$) also enabling analysis by MALDI-ToF-MS.

The objective of the current work is to demonstrate that NP-TGIC and NP-isothermal interaction chromatography (IIC) offers a unique opportunity to identify and accurately analyse a variety of high molecular weight, end-functionalised polymers by varying solvent polarity, stationary phase and temperature. Moreover, it will be shown that normal phase interaction chromatography enables the quantitative analysis of the degree of chain-end

functionality of polymers with molecular weights at which other techniques such as NMR and MALDI are capable of qualitative analysis at best. Thus, using a combination of normal phase (isothermal and temperature gradient) interaction chromatography, we report the characterisation of a series of polystyrene samples with identical molar mass (c. 90,000 $gmol^{-1}$) carrying a single chain-end functionality of varying polarity, modified by a series of end-group transformation reactions. We also describe the analysis, by a combination of normal phase (isothermal and temperature gradient) interaction chromatography, of a series of linear polybutadiene samples of varying molar mass (28,000 – 200,000 $gmol^{-1}$) synthesised by living anionic polymerisation and characterised by the presence of a single primary alcohol functionality at the chain-end.

**Experimental Section**

**Measurements.** $^1$H-NMR spectra were measured on Varian VNMRS 700 MHz spectrometer using $CDCl_3$ as solvent.

Triple detection size exclusion chromatography (SEC) was used for the analysis of molecular weight and molecular weight distribution of the polybutadiene and polystyrene samples, using a Viscotek TDA 302 with refractive index, right angle light scattering (RALS – 690 nm) and viscosity detectors and two PLgel 5 μm mixed C columns (300 x 75 mm). Tetrahydrofuran was used as the eluent at a flow rate of 1.0 ml/min and at a temperature of 35 °C. The calibration was carried out with a single narrow dispersity polystyrene standard purchased from Polymer Laboratories. A value of 0.185 mL/g (obtained from Viscotek) was used as the dn/dc for polystyrene both for the calibration and the analysis of prepared polymers. A dn/dc value of 0.124 mL/g (measured in-house) was used for the molecular weight analysis of polybutadiene.

Temperature gradient and isothermal interaction chromatography analysis was carried out using normal phase conditions. The column utilised was a diol modified silica (Nucleosil 100Å pore, 250 x 4.6 mm I.D., 5 μm) and the nature of the eluent was varied depending on the polymer analysed. A mixture of THF/isooctane (Fisher, GPC and HPLC grade respectively) was used in a ratio 45/55 (v/v) for polystyrene samples. For the polybutadiene samples a mixture of isooctane/THF was used in a ratio 96/4 (v/v). The flow rate was set to 0.5 ml/ min or 1 ml/min as required. The polymer solution concentration of TGIC samples was about 2.5 mg/ml dissolved in the eluent mixture and the injection volume was 100 μl. A modified Viscotek TDA 301 was used for the IC analysis and the detectors mainly utilised were the RALS detector and a Viscotek UV 2600 detector set to a wavelength of 260 nm for polystyrene and 215.1 nm for polybutadiene samples. The temperature of the column was controlled by a ThermoScientific circulating bath and a thermostat.

**Polystyrene synthesis.** The polystyrene samples were synthesized by living anionic polymerization using standard high vacuum techniques as previously reported[8]. This previous report describes the synthesis of the polymer along with end-group transformations from protected primary alcohol to deprotected primary alcohol to bromide functionality.

PS $M_n$= 89900 $gmol^{-1}$, $M_w$= 92400 $gmol^{-1}$, Đ 1.03

**Polybutadiene synthesis.** The end-functionalized polybutadiene samples were synthesised by living anionic polymerisation using standard high vacuum techniques. Linear polybutadiene samples with a single primary alcohol functionality at one chain-end were synthesised in benzene using sec-BuLi as initiator and the chain-end functionality was introduced via end-capping reaction of the living polymer with ethylene oxide according to a previously reported method[19].

## Results and Discussion

The analysis of end-functionalised PS and PBd polymers was carried out by a combination of normal phase isothermal interaction chromatography (NP-IIC) and normal phase temperature gradient interaction chromatography (NP-TGIC) in order to study the effect of chain-end functionalities on the IC elution and investigate the chemical heterogeneity (degree of end-capping) of end-functionalised polymers. Working under normal phase conditions is most suitable for this kind of investigation since the polar stationary phase is sensitive to the functional groups present on a polymer chain and the strength of interaction between functional group and stationary phase can be tuned by both solvent polarity and temperature. The following discussion will describe the characterisation of PS samples with the same molar mass (c. 90,000 $gmol^{-1}$) but with different chain-end functionalities, i.e. protected primary alcohol, primary alcohol and bromide functional groups. The analysis of a series of PBd samples with a single primary alcohol functionality at one chain-end and varying molecular weight were also analysed with a view to establishing the limitation of NP-IIC and NP-TGIC in terms of molecular weight for the quantitative analysis of end-functionalized polymers.

**Chain-end functionalised polystyrene.** The analysis of polystyrene end-capped with chain-end functionalities of different polarity was achieved by NP-IIC using a mobile phase comprising of a mixture of isooctane/THF (55/45 v/v) as previously described by Chang[16]. The samples analysed in this work are linear PS with same molar mass ($M_n$ 90,000 $gmol^{-1}$, Đ 1.03) but with three different chain-end functionalities, i.e. protected primary alcohol (-OX, where X is the protecting group *tert*-butyldimethylsilyl group), deprotected primary alcohol (-OH) and alkyl bromide (-Br). The samples are named PSY where Y denotes the functional group at the polymer chain-end, see figure 2.

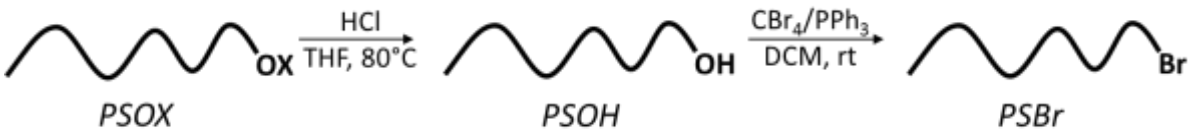



**Figure 2.** Schematic representation of functionalised PS samples including conditions required for end-group transformations[8] (PSOX - X = *tert*-butyldimethylsilyl group).

The details of PSOX synthesis and the end-group transformation reactions have been previously reported[8]. The approach used to characterise the polystyrene samples involved normal-phase interaction chromatography analysis at a fixed temperature. Thus, instead of applying a temperature gradient during the analysis, the temperature was maintained at 22°C for the entire duration of the measurement. The resulting NP-IIC analysis of the functionalised polystyrene samples under the chosen conditions of mobile phase, stationary phase and temperature resulted in the chromatograms shown in figure 3.

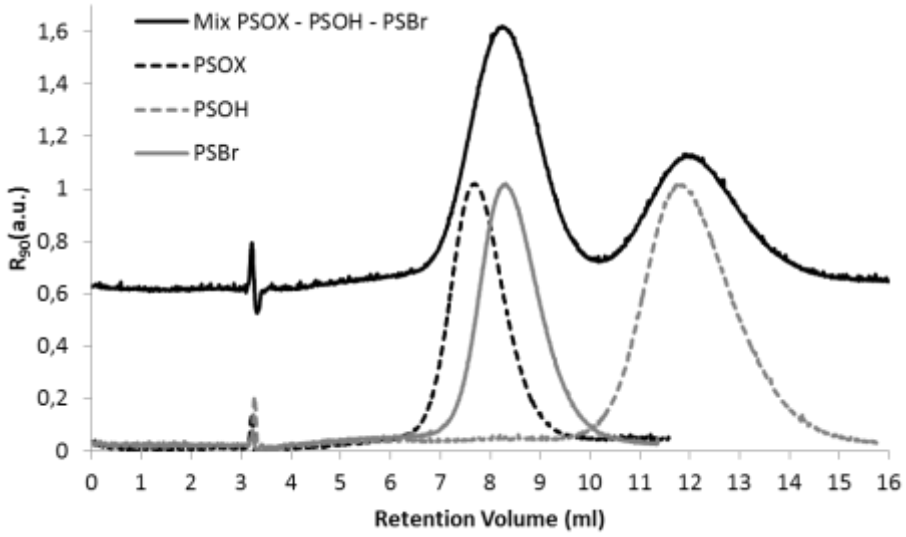



**Figure 3.** NP-IIC chromatograms (at 22°C) obtained from the analysis of PSOX, PSOH and PSBr and a mixture of PSOX, PSOH and PSBr. Chromatograms recorded by RALS detector. Solvent/injection peak at 3.3 ml of retention volume.

The data shown in figure 3 confirms that each end-functionalised polystyrene sample is eluted in IC mode, i.e. after the solvent/injection peak (at 3.3 ml) in contrast to SEC mode where samples elute before the solvent peak. Moreover, it is clear that the change of

functionality at the chain-end of the polymer has a significant impact upon the elution behaviour of the samples and it is rather remarkable that a single functionality can play such a dominant role in controlling the elution of a polymer with a molar mass of 90,000 $gmol^{-1}$. The initial sample, PSOX, with a single *tert*-butyldimethylsilyl protected primary alcohol chain-end functionality exhibited the weakest interaction of the three samples with the stationary phase and as a consequence elutes with the lowest retention volume, 7.7 ml.

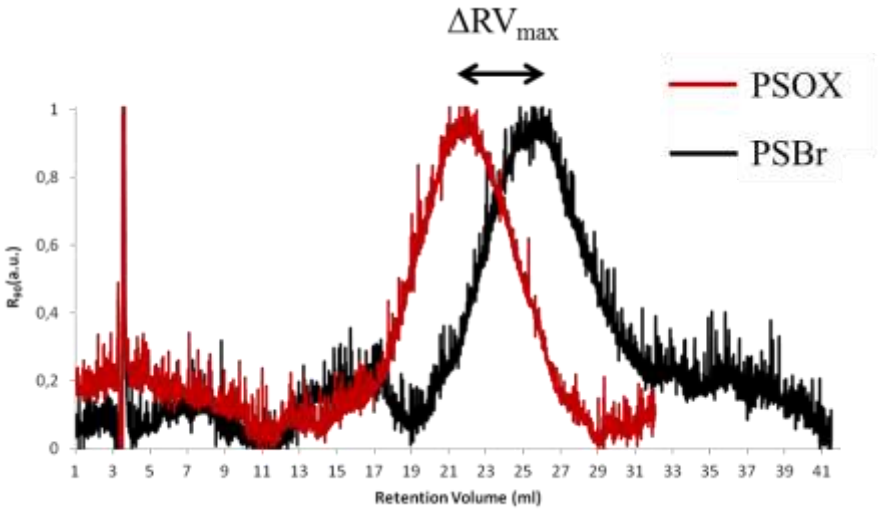



**Figure 4**. NP-IIC chromatogram at 10°C of PSOX (red) and PSBr (black) detected by RALS.

Deprotection to release the primary alcohol functionality at the chain-end of the polymer results in a much stronger interaction with the polar column packing and elution at a higher retention volume, 11.8 ml. Finally, transformation of the chain-end functionality from alcohol (PSOH) to bromide (PSBr) results in a reduced column interaction and a lower retention volume of 8.3 ml. It is worth noting that although there is a clear difference in the elution of PSOX and PSBr, the difference is not sufficient to allow the resolution of these samples during the analysis of a mixture of PSOX, PSBr and PSOH (figure 3). Yet, analysis of the same mixture shows that baseline resolution is observed between the peak attributable to PSOH and overlapping peaks of PSOX and PSBr. In an attempt to improve the resolution between PSOX and PSBr, a temperature gradient was applied during the analysis of the same mixture but the separation of the two polymers peaks was not improved. Moreover, each

individual sample (PSOX and PSBr) was analysed under NP-IIC conditions at the lower temperature of 10°C (figure 4). It might be argued that an increased separation was observed in terms of $\Delta RV_{max}$ (difference in retention volume at peak maxima) from 0.6 ml at 22° C to 3.8 ml at 10° C however the increase in resolution in terms of peak overlap is only marginal in comparison to the resolution observed at 22° C (figure 3). Although this suggests that the use of lower temperatures may be one way to somewhat improve the resolution between these two polymer samples it should also be noted that a noticeable peak broadening was observed at this lower temperature and the signal to noise ratio was substantially worse – possibly due to poorer solubility in the mobile phase at the lower temperature (figure 4).

Thus, although it has not been possible (yet) to develop conditions to obtain baseline resolution between each and every chain-end functionalised polymer in this series of polystyrene samples, the principal objective of this work was to develop a methodology to monitor a series of chain-end functional group transformations (PSOX → PSOH → PSBr) and to quantitatively analyse the efficiency of each transformation. This has been shown unequivocally to be the case. Usually, the conversion of such functional group transformations would be analysed using $^{1}$H-NMR and it is possible to make some qualitative observations about the efficiency of the reactions where the molecular weight of the polymers is modest, as has been previously reported[20] for a similar series of transformations (PSOX → PSOH → PSCl) of chain-end functionalised polystyrene with a molar mass of 12,400 $gmol^{-1}$. However, even at these modest molecular weights, accurate quantitative analysis is hampered by signal to noise ratios. At higher molecular weights this problem is significantly exacerbated due the increasingly weak signals arising from the end-groups. Therefore the analysis of high molecular weight chain-end functionalised polymers by NMR gives information only at a qualitative level and the efficiency of end-group transformations can only be based on the appearance and/or disappearance of the peaks arising from the -C<u>H</u>$_2$

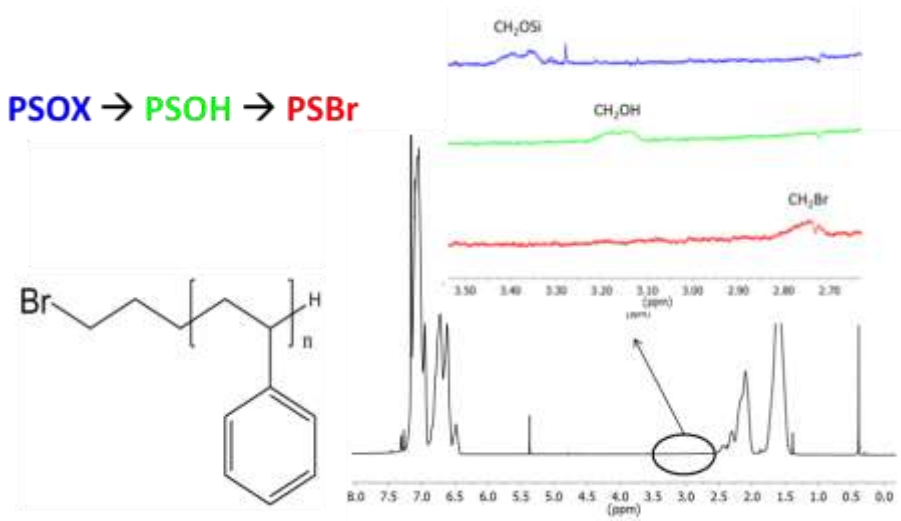



**Figure 5.** $^{1}$H-NMR analysis of PSBr (90,000 gmol$^{-1}$) and inset, analysis of end-group transformation from PSOX → PSOH → PSBr

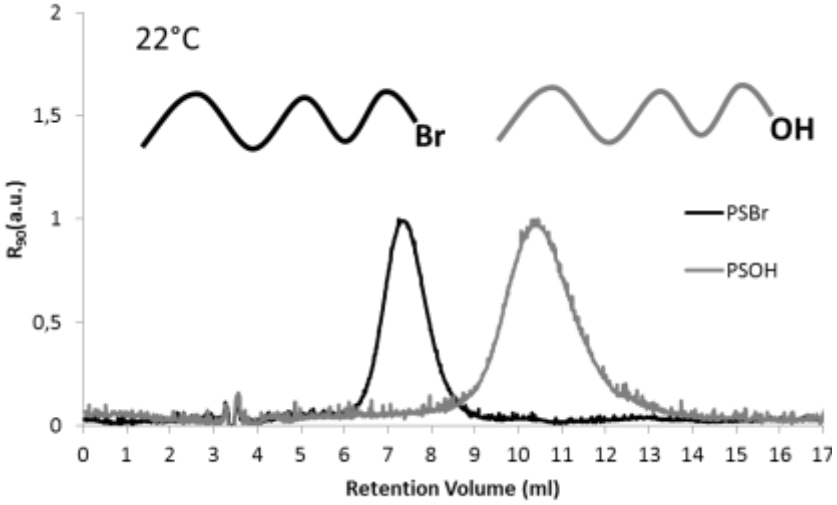



**Figure 6**. NP-IIC chromatogram at 22°C of PSOH and PSBr detected by RALS

group adjacent to the functionality in question (figure 5). Integration of these signals may be possible but errors are potentially high. In contrast the polymer peaks observed in figure 3 and discussed above are in fact easily detected and analysis of relative peak areas is facile and accurate. It is possible to observe from these IC analyses that the functional group transformation was quantitative in each case. This is revealed for the transformation of PSOX → PSOH in figure 3 where the chromatogram for PSOH shows no evidence at all of unreacted precursor PSOX and the total absence of the peak corresponding to the starting

functional polymer. The same conclusion can be drawn for the transformation of PSOH → PSBr. Although the chromatogram of PSBr in figure 3 only extends to a retention volume of 11.5 ml and thus not far enough for a claim of quantitative conversion to be made, the same two samples were analysed on another day under nearly identical conditions and this data is shown in figure 6 and it is clear from this data that conversion (PSOH → PSBr) is quantitative.

**Chain-end functionalised polybutadienes.** A series of linear polybutadiene (PBd) polymers with a single primary alcohol, chain-end functionality (PBd-OH) was synthesised and characterised by NP-IIC and NP-TGIC using a mixture of isooctane/THF (96/4 v/v) as mobile phase. The amount of THF in the mobile phase was decreased dramatically in comparison to that used for the PS samples described above (THF 45% v/v) in accordance to the very non-polar character of PBd. Such a polymer requires a mobile phase with low polarity in order to establish the optimised enthalpic interactions between the polymer and the stationary phase. The end-functionalised PBd-OH samples were analysed and compared to a series of PBd samples with the same molecular weight but lacking the alcohol end-group. The polymers were prepared by living anionic polymerisation (scheme 1) which allowed the sampling of the product before the end-capping reaction with ethylene oxide to introduce the hydroxyl group at the end of the polymer chain[19]. The unfunctionalised sample was collected from the polymerisation and terminated with methanol. The list of the PBd samples synthesised and analysed in this study is reported in Table 1. The polymers are named PBdX or PBdX-OH which indicate respectively the non-functionalised and functionalised polybutadiene and where X indicates the (approximate) weight average molar mass of the functionalised polymer.

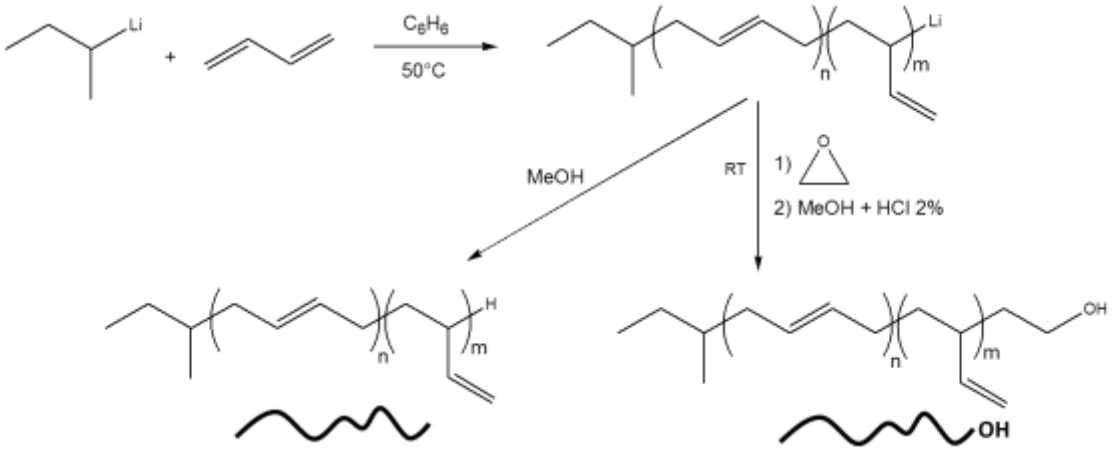
The extent of end-capping by ethylene oxide is expected to be high due to the straight forward reaction of the ethylene oxide with the living polybutadiene polymer chains.

However, the end-capping reaction did not go to completion in all cases. It is not possible to accurately calculate the exact proportion of functionalised chains in each sample by $^{1}$H-NMR due to the weak signal given from the protons (-C<u>H</u>$_2$) of the alkyl group next to the primary alcohol. An example of the vanishingly small NMR peaks observed for the highest molecular sample can be observed with the $^{1}$H-NMR analysis of PBd200-OH shown in figure 7.

**Table 1.** Molar mass data for the polybutadiene samples obtained by SEC. Triple detection calibration with dn/dc of PBd in THF (0.124). Percentage of functionalised polymer present in the final product.

| Sample | $M_n$ (g/mol) | $M_w$ (g/mol) | Đ | Functionalization (%)[(a)] |
|---|---|---|---|---|
| PBd30 | 28,600 | 29,400 | 1.03 | - |
| PBd30-OH | 28,400 | 29,700 | 1.05 | 96 |
| PBd70 | 67,200 | 70,600 | 1.02 | - |
| PBd70-OH | 68,200 | 70,000 | 1.03 | 72 |
| PBd140 | 133,700 | 136,800 | 1.02 | - |
| PBd140-OH | 134,800 | 138,200 | 1.03 | 85 |
| PBd160 | 148,200 | 158,700 | 1.06 | - |
| PBd160-OH | 148,100 | 153,200 | 1.03 | 81 |
| PBd200 | 193,900 | 201,700 | 1.04 | - |
| PBd200-OH | 197,200 | 203,100 | 1.03 | 52 |

(a) Percentage of functionalized polymer formed during the end-capping reaction with ethylene oxide calculated by deconvolution of the IC chromatograms (RI and/or UV detectors) using a Gaussian distribution.




**Scheme 1.** Reaction scheme for the synthesis of functionalised and non-functionalised PBd.

As can be clearly seen (or not) from the $^{1}$H-NMR spectrum of PBd200-OH and the polystyrene samples in figure 5, the use of NMR for the characterisation of chain-end functionality on high molecular weight polymers is not very helpful in quantifying the

success of the end-capping reaction. In contrast, analysis carried out by NP-IIC, as described above for samples of end-functionalised polystyrene, has been shown to be a powerful alternative to NMR spectroscopy for such polymers. The sensitivity of the NP-IIC system toward the presence of polar groups on the polymer chain-end allows the separation of functionalised and non-functionalised polymers, however, accurate analysis of the relative

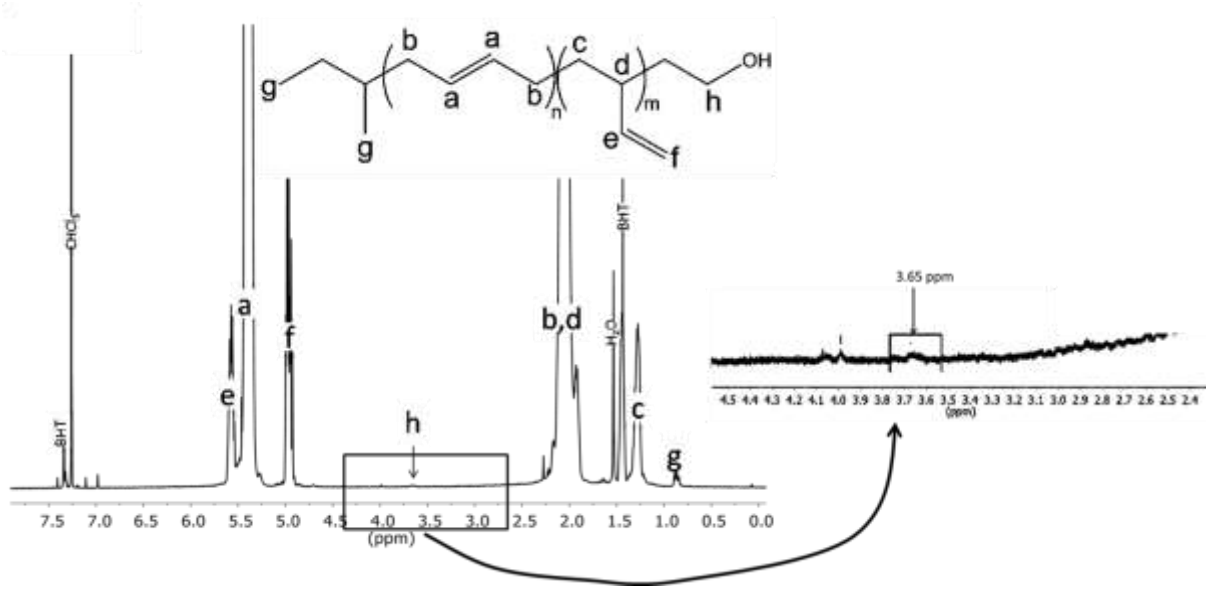



**Figure 7.** $^{1}$H-NMR (700MHz in $CDCl_3$) of PBd200-OH. Expansion in correspondence of peak h, i.e. proton signal for –C$\underline{H}_2$ next to the primary alcohol functionality.

amounts of functionalised and unfunctionalised polymer is possible by detection of the polymer chain rather than the functional group. In the case of sample PBd200-OH, the NP-IIC analysis resulted in a chromatogram characterised by the presence of two well-separated peaks (see figure 8a). Polymers PBd200 and PBd200-OH were analysed under isothermal conditions (50 °C) and the two chromatograms are here compared. The non-functionalised polymer, PBd200 shows a single peak at 5 ml, eluted soon after the solvent/injection peak (ca. 3.8 ml) whereas the functionalised polymer, PBd200-OH, shows two peaks, at 5 ml and ca. 15 ml, corresponding respectively to the non-functionalised and functionalised polymer in the polymer sample and indicating incomplete end-capping. Thus, the separation of functionalised polymer from non-functionalised polymer is possible even if the two polymers

differ only by the presence (or absence) of a single functional group at the polymer chain-end. A similar analysis was performed on sample PBd160-OH (figure 8b) which shows a

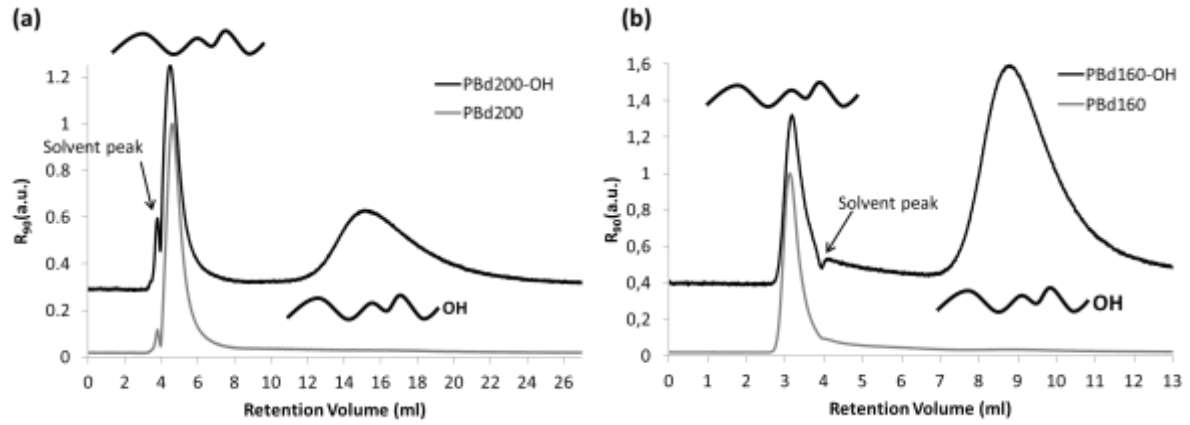



**Figure 8.** Isothermal (50°C) IC chromatograms (RALS detector): (a) PBd200 and PBd200-OH chromatograms overlaid, (b) PBd160 and PBd160-OH chromatograms overlaid. Flow rate: 0.5 ml/min. Mobile phase: Isooctane/THF (96/4). Solvent/injection peak at ca. 3.8 ml.

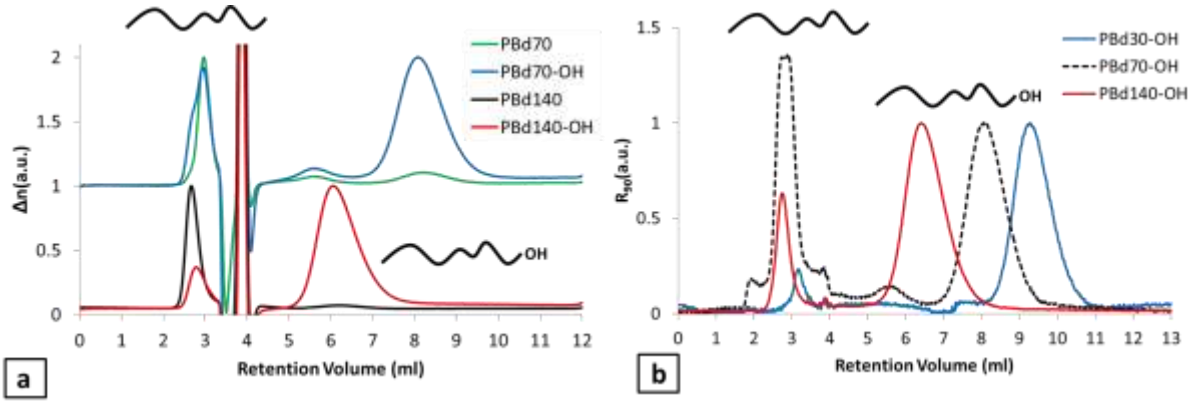



**Figure 9.** (a) Isothermal (50°C) IC chromatograms (RI detector) of PBd70, PBd70-OH, PBd140 and PBd140-OH, (b) Isothermal (50°C) IC chromatograms (RALS detector) of PBd functionalised polymers with different molecular weights. Flow rate 1 ml/min. Mobile phase: isooctane/THF (96/4 v/v). Solvent/injection peak at ca.3.8 ml.

similar result in so much that the end-capped sample, PBd160-OH revealed two peaks, one corresponding to unfunctionalised polymer at 3.5 ml and the other, functionalised polymer at about 9.0 ml. The isothermal analysis of these two functionalised polymers clearly shows that the end-capping reaction did not go to completion and the content of functionalised polymer in the samples (table 1) can be calculated in a much more accurate way by using NP-IIC chromatograms than $^{1}$H-NMR. The remainder of the samples listed in Table 1 were also

analysed under similar conditions, with the exception that a flow rate of 1 ml/min was used rather than 0.5 ml/min. The isothermal IC chromatograms obtained are shown in figure 9. Figure 9a shows the chromatograms of the functionalised samples PBd140-OH, PBd70-OH and the corresponding unfunctionalised polymers PBd140 and PBd70. It can be seen that in each case the polymers synthesis did not yield a quantitatively end-functionalised polymer, as already seen for the previous two PBd samples in figure 8. The fraction of functionalised polymer in each sample can be calculated by deconvolution of the isothermal IC chromatograms using a Gaussian distribution and the data are reported in table 1. The refractive index (RI) and the UV detector were chosen as concentration detectors to calculate the amount of the functionalised polymers. As an aside, it interesting to note that each of the unfunctionalised polymers described herein (PBd200, PBd160, PBd140, PBd70 and PBd30) elute in close proximity to each other and very close to the solvent injection peak. In the case of PBd200 the peak in figure 8a appears just after the solvent peak whilst in all other cases the peak appears just before the solvent peak. This would suggest that under the described experimental set-up the unfunctionalised polymers are very close to critical conditions at which it would be expected that all polybutadiene samples would elute at the same time irrespective of molecular weight. The fact that the elution behaviour of the unfunctionalised polymers is not entirely coincidental suggests that although close to critical conditions, these do not exactly represent critical conditions and there is still a molecular weight affect. Thus unfunctionalised polymers with a molar mass up to 160 000 $gmol^{-1}$ elute before the solvent peak, in SEC mode and they elute in SEC order with higher molecular polymer eluting before lower molecular weight polymer. However the highest molecular weight unfunctionalised polymer – PBd200 – is just sufficiently larger in molecular weight, to enhance adsorption to the column and to move this polymer into the IC regime.

It is also worth noting that figure 9a reveals further useful information about the samples. The chromatograms of PBd140 and PBd70 each contain a small second peak at 6.1 ml (PBd140) and 8.2 ml (PBd70). These small additional peaks are found in the same position as the peaks observed for the analogous functionalised polymers PBd140-OH and PBd70-OH. The origin of these peaks in the non-functionalised polymers samples can be explained by unwanted side reactions as a result of the presence of traces of air during the termination step, which can introduce of a variety of chain-end functionalities as a result of reaction between the propagating carbanion and moisture, oxygen and/or carbon dioxide[21]. Therefore, the nominally unfunctionalised polymers may also contain polymer chains with polar end-groups that elute at a similar retention volume to the functionalised PBdX-OH samples. Another possible consequence of termination due to environmental impurities is chain coupling, leading to a doubling of the molecular weight of the polymer sample[21]. Evidence of chain coupling is particularly evident in the chromatogram of PBd70-OH, in both figure 9a and 9b. In figure 9a the peak at c. 3 ml retention volume, corresponding to the unfunctionalised polymer present in sample PBd70-OH, has a clear and prominent shoulder to lower retention volumes. Since this polymer is eluting before the solvent peak, it is in SEC mode and the shoulder at lower retention volumes will be of higher molecular weight. The same peak also appears to be bimodal when detected by RALS (figure 9b). The fact that the "shoulder" detected by the RI (concentration) detector (figure 9a) has grown in intensity when detected by RALS (figure 9b) is entirely consistent with the assertion that the second species (shoulder) is higher in molecular weight than the main peak. Evidence of a notable level of chain-chain coupling is also clear in the SEC chromatogram of PBd70-OH, and to a lesser extent in other samples, which are included as supporting information. Figure 9b shows the overlaid NP-IIC chromatograms (RALS detector) of a series of functionalised polybutadienes (PBdX-OH) with molar masses from 30,000 to 140,000 $gmol^{-1}$. It can be seen clearly that

after end-capping with ethylene oxide, each sample contains two peaks, corresponding to functionalised and unfunctionalised polymer. Each unfunctionalised polymer is eluted before the solvent/injection peak (3.8 ml) and the functionalised polymer peak is found after the solvent peak in a range from 6.0 to 9.5 ml, with the lowest molecular weight functionalised polymer, eluting at the highest retention volume. During this study it was also noticed that decreasing the temperature at which the NP-IIC analysis was carried out had a profound effect upon the elution behaviour of both the functionalised and unfunctionalised polymer peaks. As the temperature is lowered, both peaks in PBdX-OH tend to elute at higher retention volumes – as expected – and the peak corresponding to the unfunctionalised polymer overlaps with the solvent/injection peak indicating critical conditions for the unfunctionalised polymers. The second peak, corresponding to the functionalised polymer, tends to shift to higher elution volumes, broaden significantly and eventually disappear with decreasing the temperature. Figure S11 (supporting information), illustrates this phenomenon for PBd140/PBd140-OH and shows that at 38 $^{o}$C the functionalised polymer is retained in the column due to enhanced polymer-stationary phase interactions.

A further point of interest is that under the conditions reported in figure 9 – namely 50°C and isooctane/THF (96/4 v/v) - NP-IIC analysis results in elution of the functionalised polymers *after* the solvent injection peak (IC mode), although the order of elution with respect to molecular weight would appear to be in SEC mode, i.e. the high molecular weight polymers are eluted first. Moreover, the corresponding unfunctionalised polymers elute almost simultaneously with the solvent peak and more or less independently of molecular weight, suggesting that these conditions are very close to critical conditions for (unfunctionalised) polybutadiene. Thus, although the order of elution of the functionalised polymers is consistent with separation under SEC mode (high molecular weight at low retention volumes and low molecular weight at high retention volumes) separation in this case is NOT on the

basis of hydrodynamic volume. We believe that the separation of the functional polymers of different molecular weight is entirely due to the balance of enthalpic interactions in the system. Thus, the non-polar hydrophobic solvent (isooctane/THF 96/4 v/v) is an excellent solvent for the hydrophobic polybutadiene polymer backbone which, in turn has little affinity for the polar stationary phase. However, the polar hydroxyl end-group has a strong affinity for the stationary phase. The balance of these competing interactions (adsorption versus desorption/solvation) dictate the elution behaviour. At high molecular weight the affinity of the polar -OH end-group for the stationary phase is overcome by the solvation of the polymer backbone and elution volumes are lower. At the lowest molecular weight, the contribution of end-group adsorption is stronger, retention is enhanced and elution volumes are higher. Under critical conditions for the polymer backbone, separation is determined by the relative impact of the end group on adsorption.

To further investigate the separation of OH end-functionalised polybutadiene, the analysis of samples PBd30-OH, PBd70-OH and PBd140-OH was repeated under temperature gradient interaction chromatography conditions and moreover, conditions which ensured the un-functionalised polymers were eluted after the solvent/injection peak, i.e. working in NP-TGIC conditions for polybutadiene (figure 10).

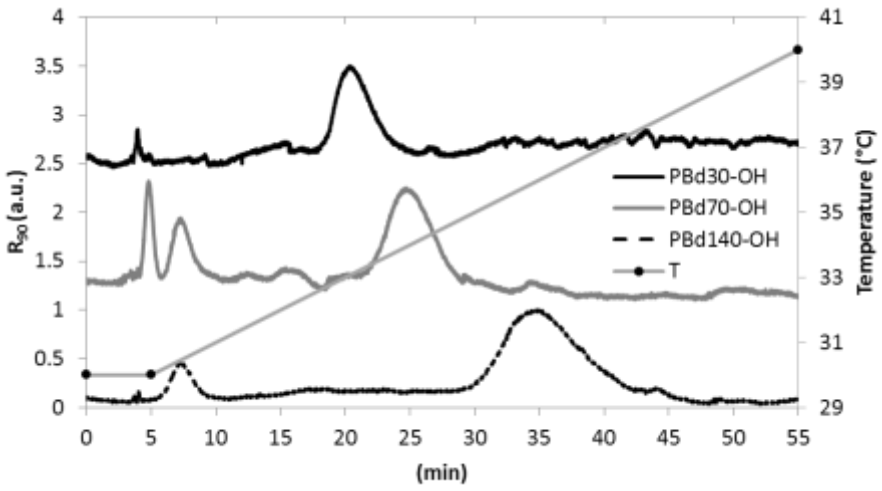



**Figure 10.** NP-TGIC chromatograms of PBd30-OH, PBd70-OH and PBd140-OH recorded with RALS detector. The profile temperature reported has a heating rate of 0.2°C/min. Solvent/injection peak is at 3.6 ml and flow rate 1 ml/min. Mobile phase: isooctane/THF (96/4 v/v).

The baseline of the chromatograms is rather noisy in the NP-TGIC chromatograms which can be an inherent problem with TGIC given the sensitivity of the detectors to changing temperature however, each of the chromatograms clearly shows the presence of one or more peaks after the solvent/injection peak at 3.6 ml, indicating that elution of the polymers is according to IC mode. The peaks of the functionalised polymer are found at high retention volumes for all the three samples and the order of elution of the functionalised polymers is now the reverse of that seen in figure 9, namely the three functionalised polymers are eluted in order of increasing molecular weight: PBd30-OH, PBd70-OH and finally PBd140-OH. This order of elution is also consistent with the single previous example of NP-TGIC analysis of end-functionalised polymers reported by Chang[16] (figure 1). It has been previously calculated from the NP-IIC analyses that the hydroxyl-functionalised polymers each contain a certain proportion of unfunctionalised chains (table 1). In the case of PBd30-OH, it was shown that about 96% of this polymer is functionalised and the only significant peak is eluted at 20 ml. PBd140-OH is only 85% functionalised and the peak at low retention volume (7.3 ml) representing the non-functionalised polymer chains is evident. In the case of PBd70-OH which is 72% functionalised, three peaks are visible in the TGIC chromatogram. The first

peak at 4.7 ml can be attributed to the unfunctionalised polymer and the peak at 24.6 ml can be attributed to the functionalised polymer. We believe that the peak at 7.3 ml represents polymer chains with double the molar mass of the main polymer – i.e. double 70,000 $gmol^{-1}$ – which arises from polymer-polymer coupling due to reaction with environmental impurities during the termination step of the living anionic polymerisation, as alluded to early. SEC analysis of this sample (figure S4 – supplementary information) confirms the presence of this impurity in the polymer. The elution of this peak at approximately 7.5 ml appears after the peak at 4.7 ml for PBd70 and at almost the same retention volume as PBd140 (7.3 ml), is entirely consistent with the fact that this peak represents coupled polymer chains with double the molecular weight of PBd70. It is nonetheless worth noting again that it is interesting that the order of elution of the functionalised polymers switches when we move from (nearly) critical conditions to IC conditions for (unfunctionalised) polybutadiene.

**Conclusions**

We have described here the characterisation of two classes of chain end-functionalised polymer (polystyrene and polybutadiene) by normal phase interaction chromatography (NP-IIC) and normal phase temperature gradient interaction chromatography (NP-TGIC). The analyses were carried out in order to follow a series of end-group modification reactions and study the chemical heterogeneity/degree of end-capping of high molecular weight polymers samples synthesised by living anionic polymerisation. NP-IIC and NP-TGIC are shown to be powerful tools for such analyses and uniquely enables the accurate analysis of such samples at very high molar mass (greater than 200,000 $gmol^{-1}$). The analysis of such polymers using more common techniques such as NMR or MALDI ToF MS is at best qualitative and often of little use at all.

NP-IIC analysis of a series of polystyrene samples with the same molar mass (90,000 gmol$^{-1}$) but decorated with different chain-end functionalities, resulted in chromatograms with clearly resolved peaks. It was shown that the polarity of the functional group dictates the elution volume of the polymers and that NP-IIC could be used to monitor the quantitative end group transformation from silyl-protected alcohol → primary alcohol → alkyl bromide. Thus, instead of estimating the success of the conversion reaction from the very small peaks observed in $^{1}$H-NMR spectra, NP-IIC gives clear chromatographic peaks, whose retention volumes change dramatically only as a consequence of each new chain-end functional group.

A series of polybutadiene samples with differing molecular weight, but each functionalised with a single primary alcohol group at one chain-end, were also analysed by NP-IIC. The primary alcohol functionality enabled the separation of functionalised and non-functionalised polymers, which eluted at different retention volumes despite identical molecular weights. For each functionalised sample, the presence of unfunctionalised polymers was revealed, resulting from an incomplete end-capping reaction with ethylene oxide and moreover, analysis of the area under the concentration chromatogram, allowed quantitative and accurate analysis of the extent of end-capping. It is all the more remarkable that complete separation can be achieved for two polymers of identical molar mass, over 200,000 gmol$^{-1}$, which differ only in that one sample is decorated with a single primary alcohol group. It was also shown that under the chosen isothermal conditions (mobile phase isooctane/THF (96/4 v/v) at 50°C), the functional polymers were eluted after the solvent/injection peak but in increasing order of molecular weight. It was concluded that these conditions represent (close to) critical conditions for the polybutadiene backbone and the order of elution is governed solely by the relative contribution of the polar end-group to stationary phase adsorption. This relative contribution is countered by the favourable polymer-solvent interactions (promoting desorption) which become progressively more dominant as the molecular weight of the

polymer increases, leading to weaker column adsorption and shorter elution times. True NP-TGIC order of elution is in fact found when a temperature gradient is applied to the system and IC conditions for (unfunctionalised polybutadiene) prevail. In this case the order of elution of the functionalised polybutadiene samples is reversed and the lower molecular functionalised polymer is eluted first.

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