Investigation of low molecular mass substances for their suitability as internal standards in GPC analysis together with nitrocellulose

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Abstract

For four nitrocellulose as samples and two different columns sets suitable internal standards were found for the application in gel permeation chromatography (GPC) using a refractive index detector. With column set 1 (two mixed bed columns in series) as the most suitable internal standards have been identified trinitrotoluene (TNT) and di-(n-hexyl) phthalate (DnHP), whereby a small peak appearing in the low molar mass region of the tested nitrocelluloses had to be neglected. With column set 2 (five columns with single porosities in series) di-octyl sebacate (DOS) and diphenylamine (DPA) are possible substances. DPA can be seen as best choice because of its distinctive signal in the refractive index (RI) detector.

1. Introduction

In modern gel permeation chromatography (GPC) the trend is to speed up the analytical procedures. This can be achieved by designing accessories as 'high speed' columns and also by the development of analytical procedures. One of these procedures is the use of an internal standard.

In extended analytical series it is possible that a change in the solvent flow and therefore a dislocation in retention volume V_R happens. This is counteracted by the use of a flow or retention volume marker. Therewith the fluctuations can be identified and corrected afterwards by the GPC software. Moreover another benefit of the use of a flow marker is that a daily replicated calibration with narrowly distributed polystyrene standards can be renounced. This means that a greater amount of samples can be measured per day.

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2. Facts to be considered in using an internal standard

When using a refractive index (RI) detector in GPC this detector shows several positive and negative signals at the total penetration limit of the columns, even when no sample but only pure solvent is injected. These peaks are not part of the sample, but of the overall system. This happens even when using the same solvent for elution and for desolving the sample. Therefore the peaks shown in Figure 1 are generally called solvent or system peaks.

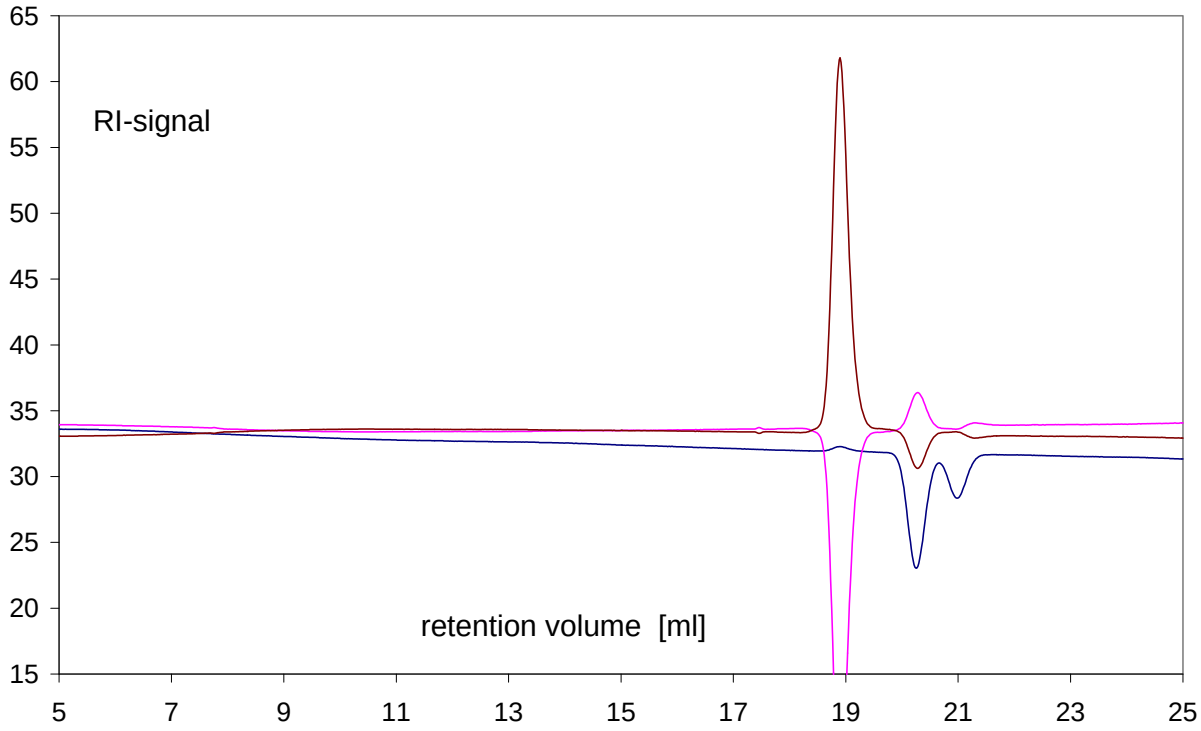

Figure 1: Three examples of the so-named solvent peaks or system peaks in the signal of the refractive index (RI) detector. The solvent used here is tetrahydrofurane (THF). The peaks can be positive or negative with regard to the baseline.

Since the system peak remains not always constant it must be ensured that an internal standard is applicable for the planed analysis. The usability of an internal standard depends on (I) the column set used for GPC analysis, on (II) the solvent (eluent), (III) on the detector used and strongly on (IV) the sample under consideration. The intensity of the refractve index detector (RI) signal of nitrocellulose is rather low in tetrahydrofuran (THF). The consequence is that NC analysis by GPC requires special attention on the behaviour and applicability of substances used as internal standards.

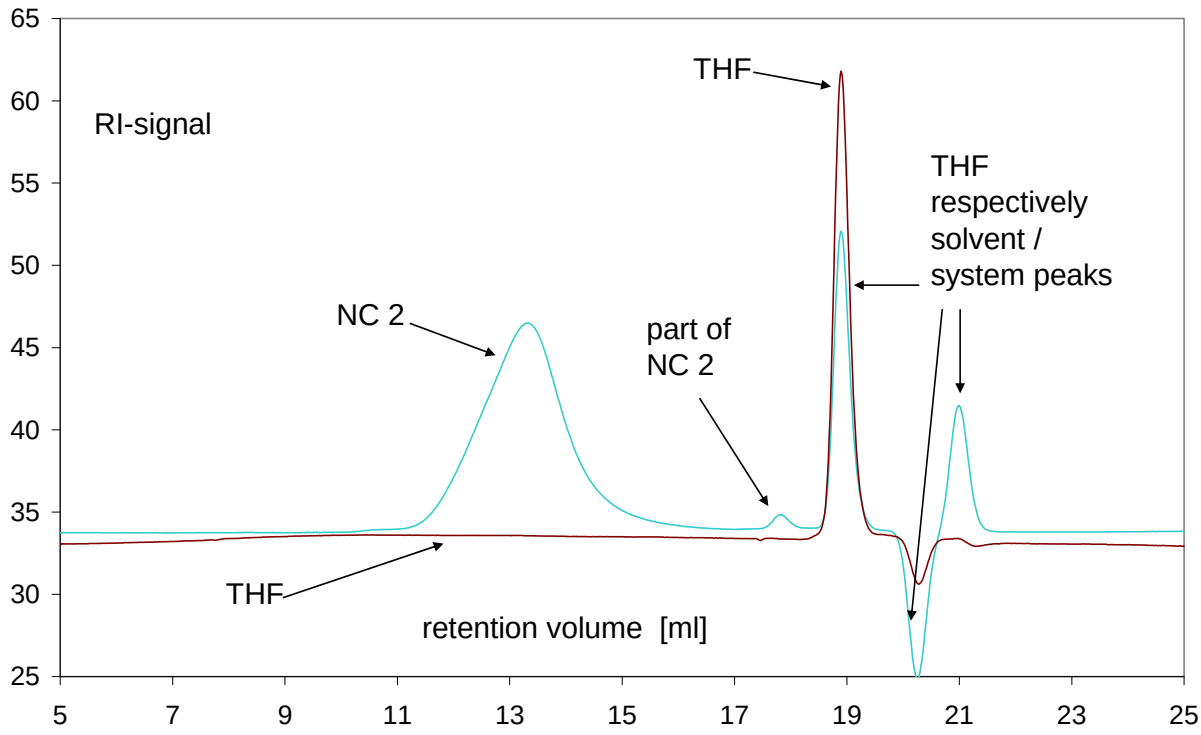

Figure 2: Elugramme of a nitrocellulose (NC) sample in THF overlapping with the system peak. The start of the system peak has to be determined by running THF as a sample (see Figure 1). The small peak at 17.9 ml retention volume belongs to the NC sample – NC molecules with small molecular mass or an additive.

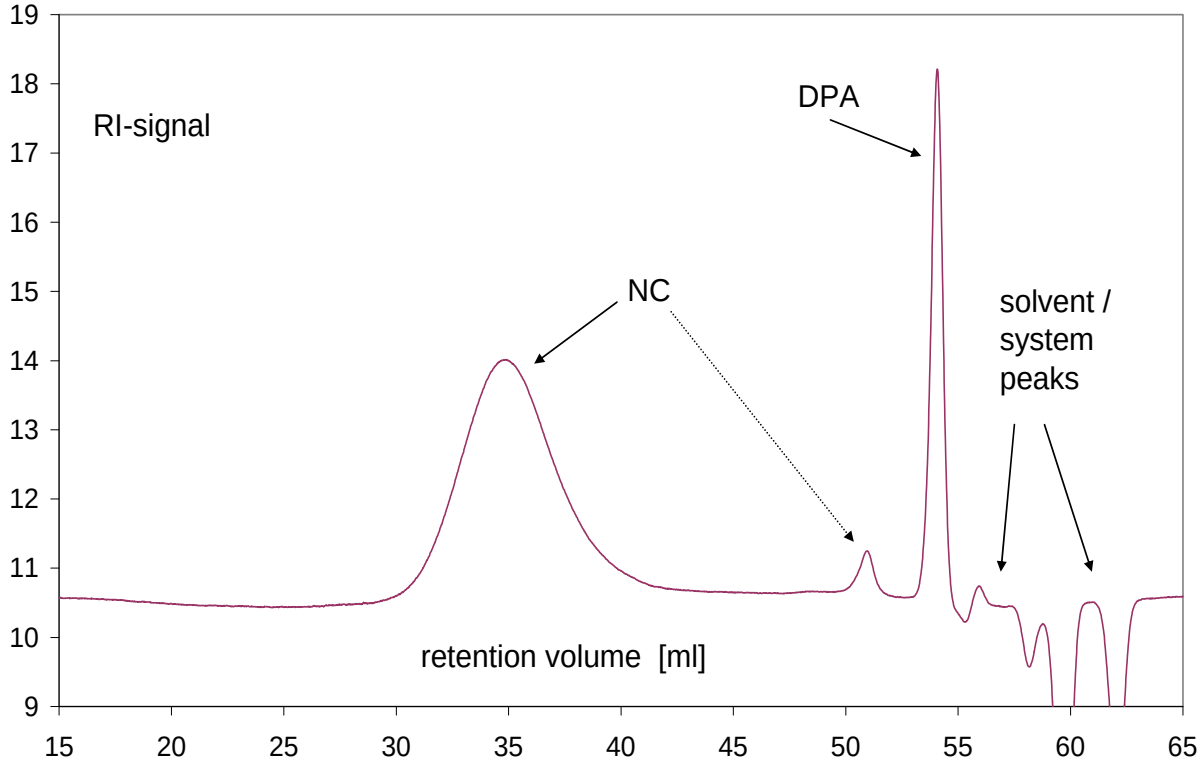

Figure 3: Elugramme of NC 2 with DPA as internal standard.

In former investigations [1] with toluene as internal standard we found a dislocation of the toluene peak in the presence of NC in comparison with toluene in THF injected alone. This strange effect occurred not with other polymer samples as polystyrene or with the calibration material. So we inferred from this observation that the behaviour of toluene was connected with the presence of nitrocellulose. The determination of the column plate numbers of the column set revealed that toluene eluates with a broader peak when NC is present. Some general wording for such a phenomenon is that nitrocellulose caused a peak impurity of toluene. However, the retention volume of toluene was changed also. Possible explanations are: (I) There is a substance inside the NC which is desorbed by toluene so it will be eluated only when toluene is in the THF-solvent; (II) Nitration of toluene by weakly bonded nitrate ester groups of NC. This nitration changes the hydrodynamic size of the molecule and the shift in retention volume is resulting.

Hence for the suitability of a substance to be usable as internal standard in NC-GPC some requirements have to be fulfilled:

- The molar mass of the internal standard has to be high enough to ensure that the internal standard eluates outside of the solvent peak or system peak.
- The molar mass of the internal standard has to be low enough to ensure that the peak of the internal standard does not coincide with an essential part of the NC peak.
- The internal standard must not be affected by the sample material, in this special case the nitrocellulose.

3. Experimental

3.1 Analytical techniques

Gel permeation chromatography was performed with instruments from the Agilent series 1100 including the RI-detector Agilent 1100. Two column sets were used: first set, named set 1: two columns in series of type Plgel 10 µm MIXED-B (mixed bed) obtained from Polymer Laboratories; second set, named set 2: a series with five columns of type SDV from company PSS, Mainz, Germany, with pore sizes 50 Å, 100 Å, 10^3 Å, 10^5 Å, 10^6 Å and with particle size of 10 µm. In both cases the columns were 8.0 mm in diameter and 300 mm long. Ten narrowly distributed polystyrene standards, of type ReadyCal from PSS, were applied to calibrate the apparatus. Their peak molar masses Mp ranged between 7500000 and 580 g/mol.
Columns temperature was 35 °C, solvent flow 1 ml/min and injection volume 100µl for both column sets.

3.2 Materials used for the analyses

The materials chosen as potential internal standards are shown in Table 1. Three nitrocelluloses with nitrogen content from 11.0 to 13.55 mass-% have been chosen for the GPC investigation:

NC 1 with a nitrogen content of 13.55 mass-%
NC 2 with a nitrogen content of 12.55 mass-%
NC 3 with a nitrogen content of 12.10 mass-%

Figure 4 shows the molar mass distributions of the NC samples.

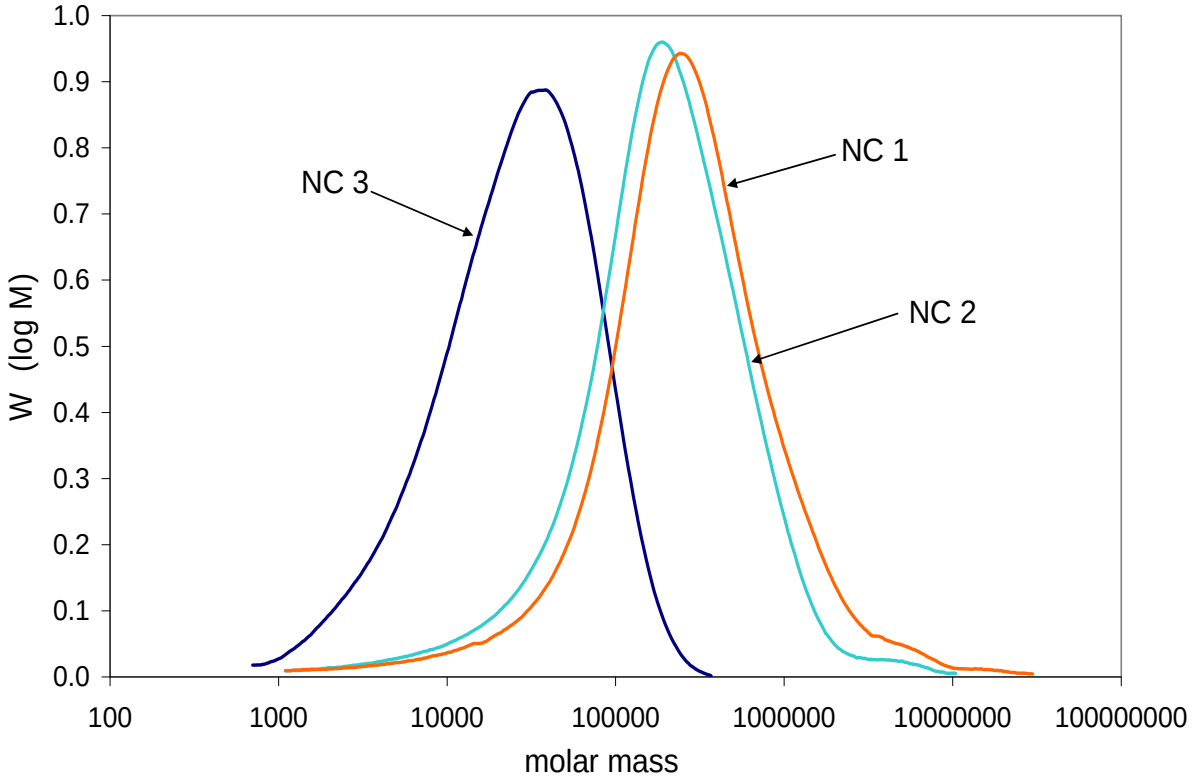

Figure 4: Molar mass distributions of three NCs chosen as samples for the measurements.

First the internal standard candidate was dissolved in tetrahydrofurane (THF) which was also the eluent in the GPC analysis. Afterwards the NC was dissolved in the solutions. After a dissolving time of 24 hours at room temperature the samples were injected in the GPC apparatus.

Table 1: Substances used as internal standards ordered by molecular mass

Substance	abbreviation	Molar mass [g/mol]
Toluene		92.14
Campher		153.23
Diphenylamine	DPA	169.23
Acardite II	Ak II	226.27
Nitroglycerine	Ngl	227.09
Trinitrotoluene	TNT	227.13
Butanetriol trinitrate	BTTN	241.11
Trimethylolethane trinitrate	TMETN	255.14
Ethyl centralite	EC	268.35
Di-(n-butyl) phthalate	DBP	278.34
Di-(n-hexyl) phthalate	DHB or DnHP	334.45
Di-octyl adipate	DOA	370.57
Di-octyl sebacate	DOS	426.67

4. Results and discussion

4.1 Suitability of a substance as internal standard together with NC and column set 1

4.1.1 Toluene

Principally it would be possible to work with toluene (molecular mass 92.14 g/mole) as internal standard since toluene produces a rather high peak at 19.5 ml (Figure 5). With the column set 1 (two Plgel 10 µm MIXED-B in series) it was possible to work with toluene as internal standard together with three of the four NCs. But for the nitrocellulose sample NC 1 with the highest nitrogen content (13.55 mass-%) we found again – as before and reported in [1] – that the toluene peak was dislocated when being in contact with NC 1 (Figure 6). For this reason toluene can be stated as substance with low molecular weight being not appropriate as internal standard together with nitrocellulose.

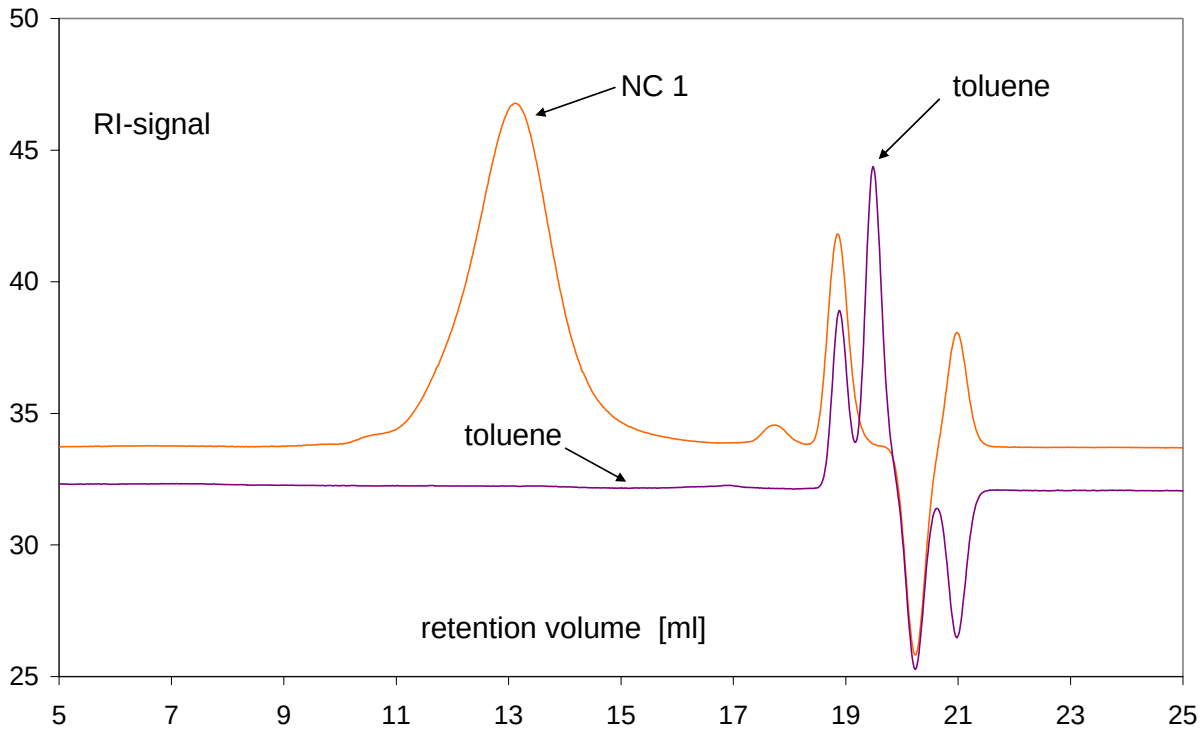

Figure 5: NC 1 in THF and 222 ppm toluene in THF. The peak at 18.9 ml is part of the solvent peak.

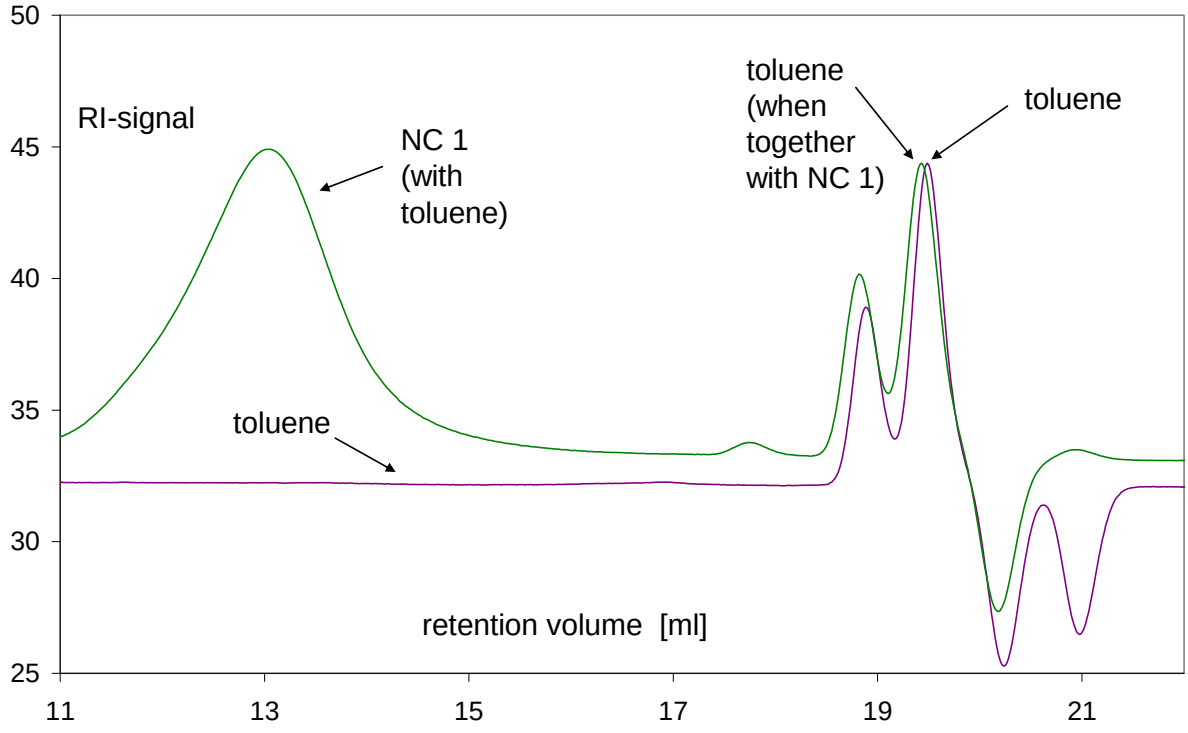

Figure 6: Peak of toluene in THF and dislocated peak of toluene being in contact with NC 1 in THF. The peak at 18.9 ml is part of the solvent peak.

4.1.2. Trinitrotoluene (TNT)

Trinitotoluene (TNT) with a molecular mass of 227.13 g/mol would be another possible internal standard for GPC analysis of energetic materials. TNT has the benefit that it cannot be nitrated by nitrocellulose.

It was found that TNT does not move when being in contact with NC. Moreover the TNT peak is intense enough for the use as internal standard, Figure 7. But it is appearing shortly after a smaller peak at 17.9 ml seeming to belong to nitrocellulose . If there is no intention to include this peak in the evaluation of nitrocellulose than the use of TNT as internal standard would be possible.

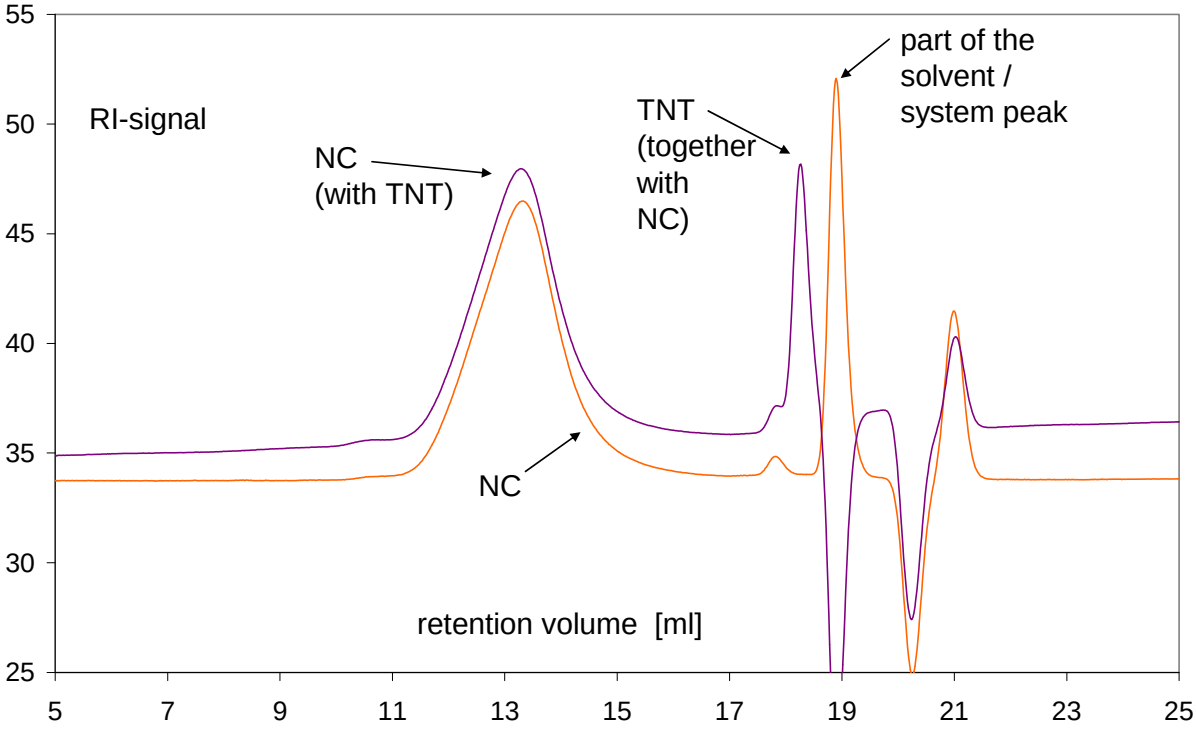

Figure 7: TNT as internal standard with the nitrocelluose NC 2. The peak at 18.9 ml is a part of the solvent peak. In the NC elugramme the solvent peak appears in the positive range, in the NC with TNT elugramme the solvent peak is directed negatively.

4.1.3 Acardite II (Ak II)

When using acardite II (Ak II) with a molecular mass of 226.27 g/mol as internal standard there is no shift in the presence of NC. But the part of the signals of the solvent or system peak beginning at 18.9 ml and of Ak II at 18.55 ml get too much interferred for a proper use of this substance as internal standard, see Figure 8 and Figure 9.

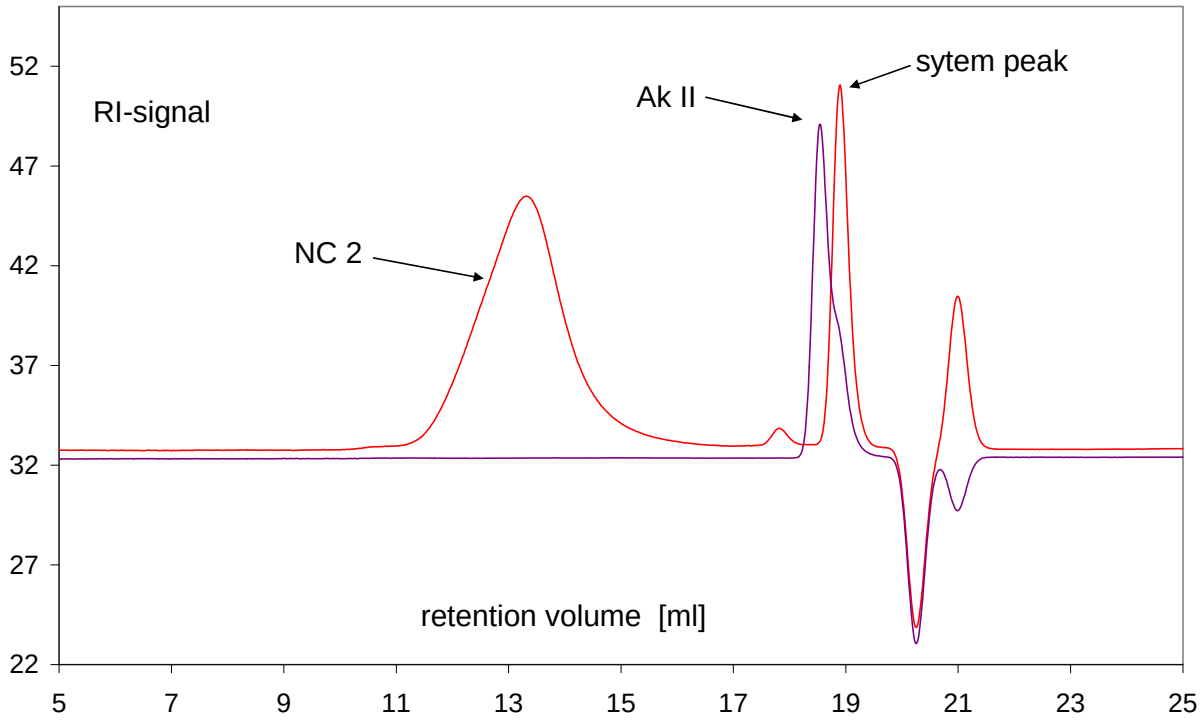

Figure 8: Elugrammes of NC 2, Ak II and THF. Ak II creates a peak at 18.55 ml, but the system peak begins already at 18.9 ml.

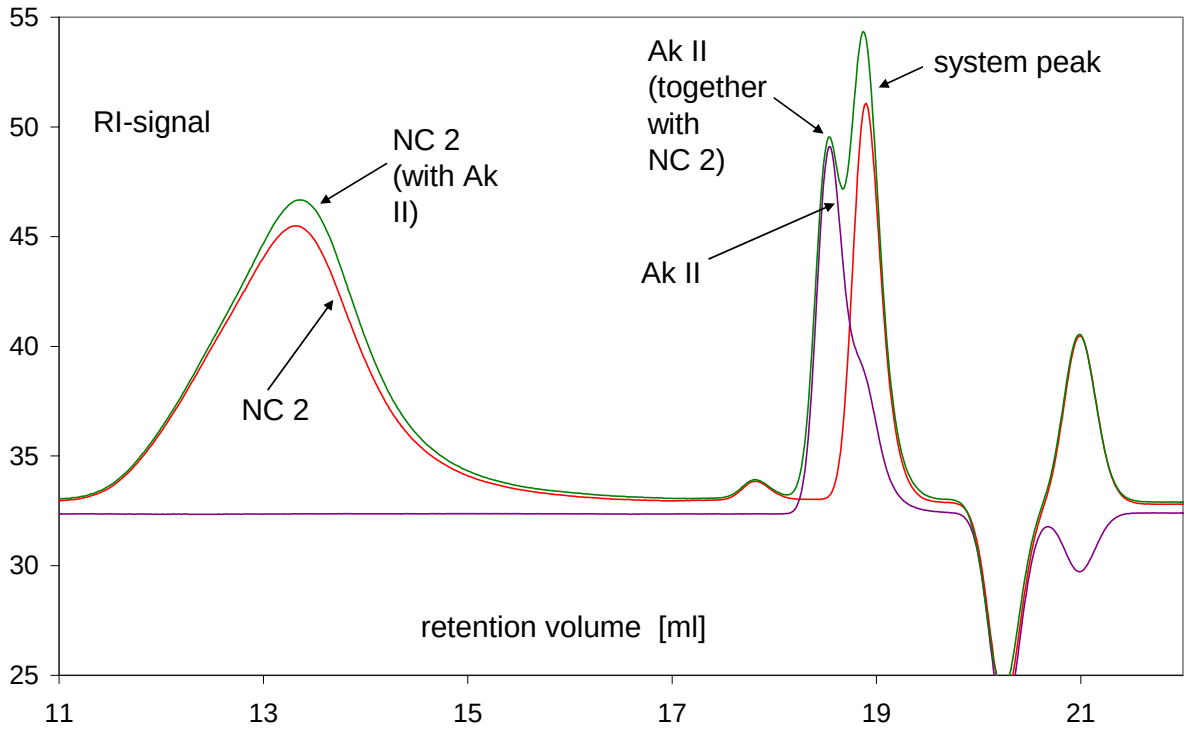

Figure 9: Elugramme of the sample NC 2 with Ak II in comparison to the elugrammes of NC 2 and Ak II alone. In the sample elugramme the system peak at 18.9 ml is blurring into the peak of Ak II.

4.1.4 Di–(n-hexyl) phthalate (DnHP)

Di-(n-hexyl) phthalate (DnHP) with a greater molecular mass than that of acardite II (Ak II: 226.27 g/mole – DnHP: 334.45 g/mole) is producing a signal which is not extending into the system peak. But because of its higher molecular mass it is overlapping with the low elugramme parts belonging to the NC sample. If this part of the sample can be neglected, DnHP could be used as a proper internal standard, Figure 10.

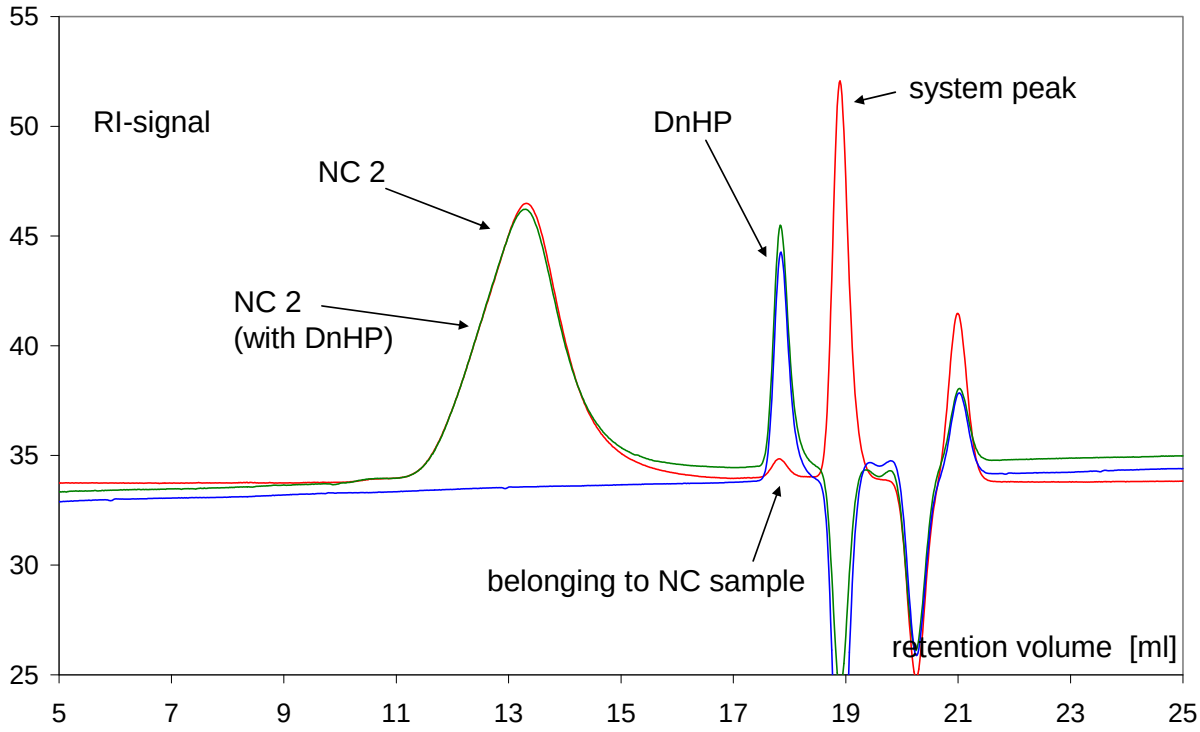

Figure 10: Elugrammes of NC 2 with DnHP in comparision with the ones of NC 2 and DnHP alone.

4.1.5 Nitroglycerine (Ngl)

Nitroglycerine (Ngl) with the molecular mass 227.09 g/mol shows a rather small peak in the RI-detector, which overlaps somewhat with the small peak part of the NC sample at higher retention volume. However, worse is that the Ngl peak is dislocated – as with toluene – when in contact with nitrocellulose. This behaviour occurred with all used NCs. Figure 11 shows this fact with NC 1.

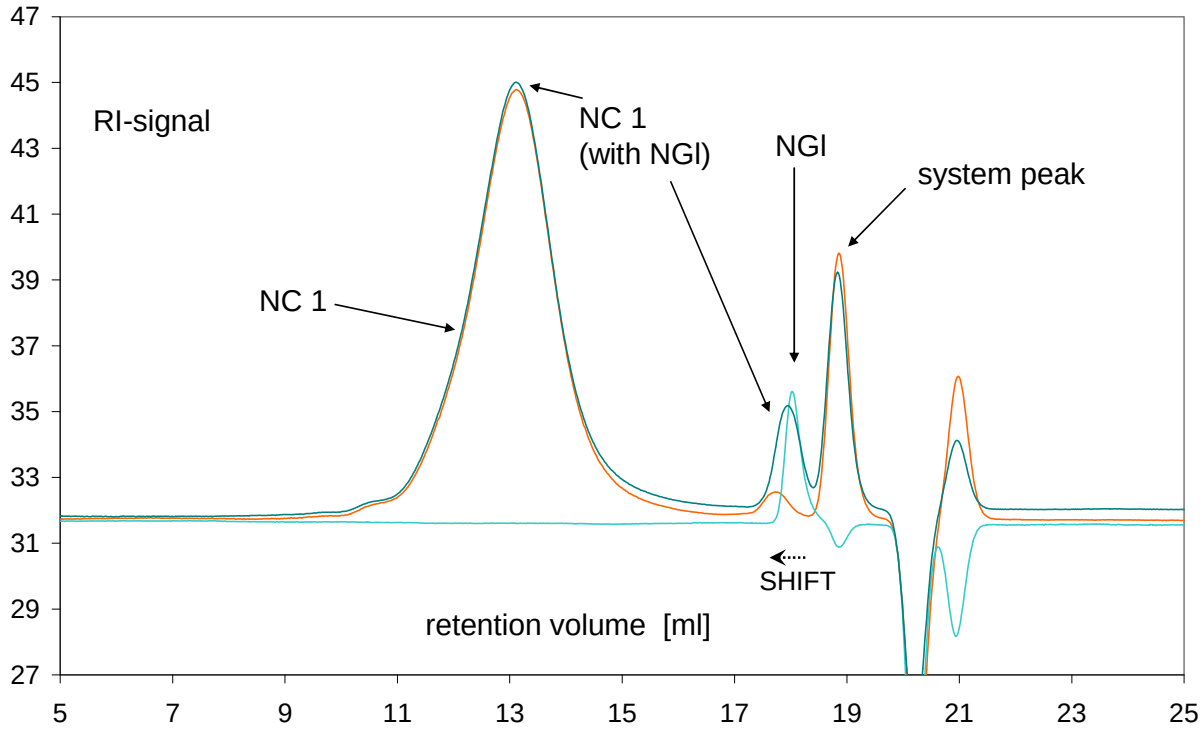

Figure 11: The Ngl-peak is shifted when Ngl is in contact with nitrocellulose.

4.1.6 Conclusion on appropriate substances as internal standards with NC when using column set 1: two times MIXED-B columns

When working with set 1, two columns in series of type Plgel 10 µm MIXED-B, and nitrocellulose, the substances toluene, Ngl and acardite II are not appropriate as internal standard. TNT and DnHP could be used as internal standard provided that the small peak at 17.9 ml (appearing with NC 1 and NC 2) will be not included in the evaluation.

A set of two columns has a small separating capacity, here especially in the range of the low molecular mass range. So the possibilities for the use of an internal standard are restricted. Moreover NC 1 shows a little 'bumb' before the main peak, means a pre-peak in the high molar mass part of the distribution, see Figure 12, indicating that the high molecular masses of the nitrocellulose are not separated by this column set. To have a look on this problem and to have more separation range another set of columns was chosen for further measurements.

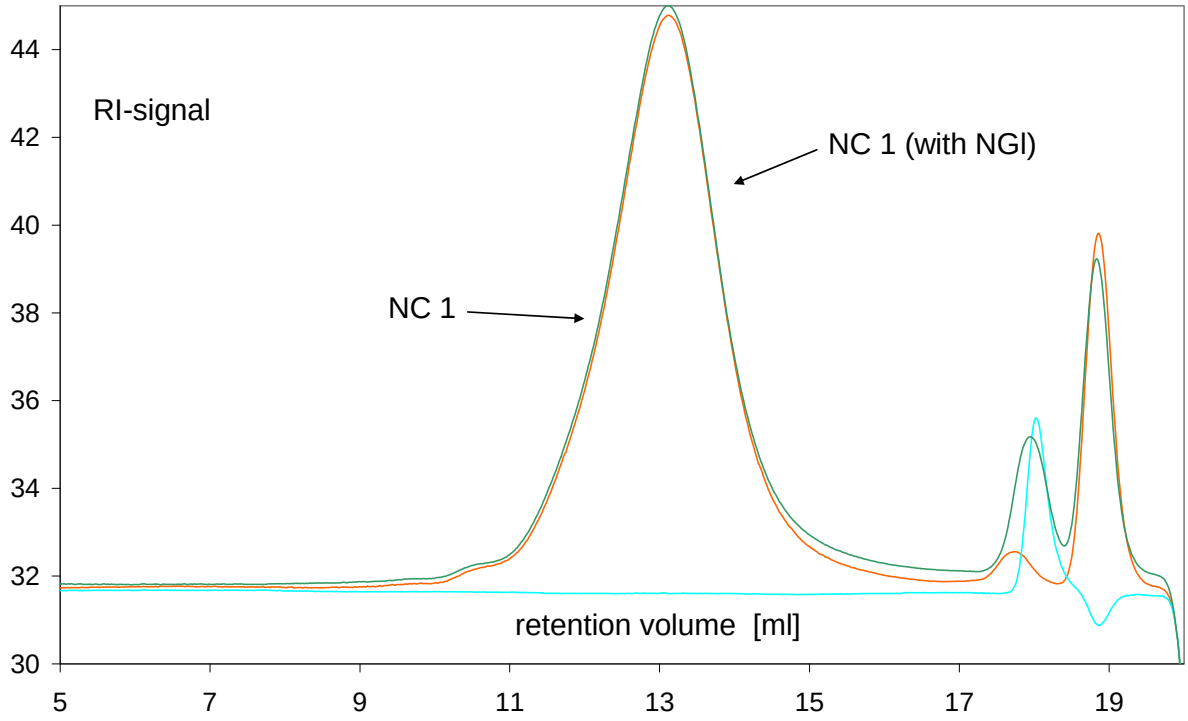

Figure 12: Small pre-peak at the beginning of the distribution with the column set 1: two Plgel 10 µm MIXED-B columns in series.

4.2 Suitability of a substance as internal standard together with NC using column set 2

4.2.1 Nitroglycerine (Ngl) and Di-(n-hexyl) phthalate (DnHP)

Nitroglycerine (Ngl) showed a shift in retention time, which made it impossible for use as internal standard. Moreover the peak of Ngl becomes indistinct from the small part of NC appearing at 51.1 ml, see Figure 13.

Di-(n-hexyl) phthalate (DnHP) has its peak at the same retention volume as the small low molar mass peak of NC. It cannot be used as internal standard if this part of the NC must be included in the evaluation. If this is not the case DnHP would be a good internal standard since it is intense enough and not shifting, Figure 13.

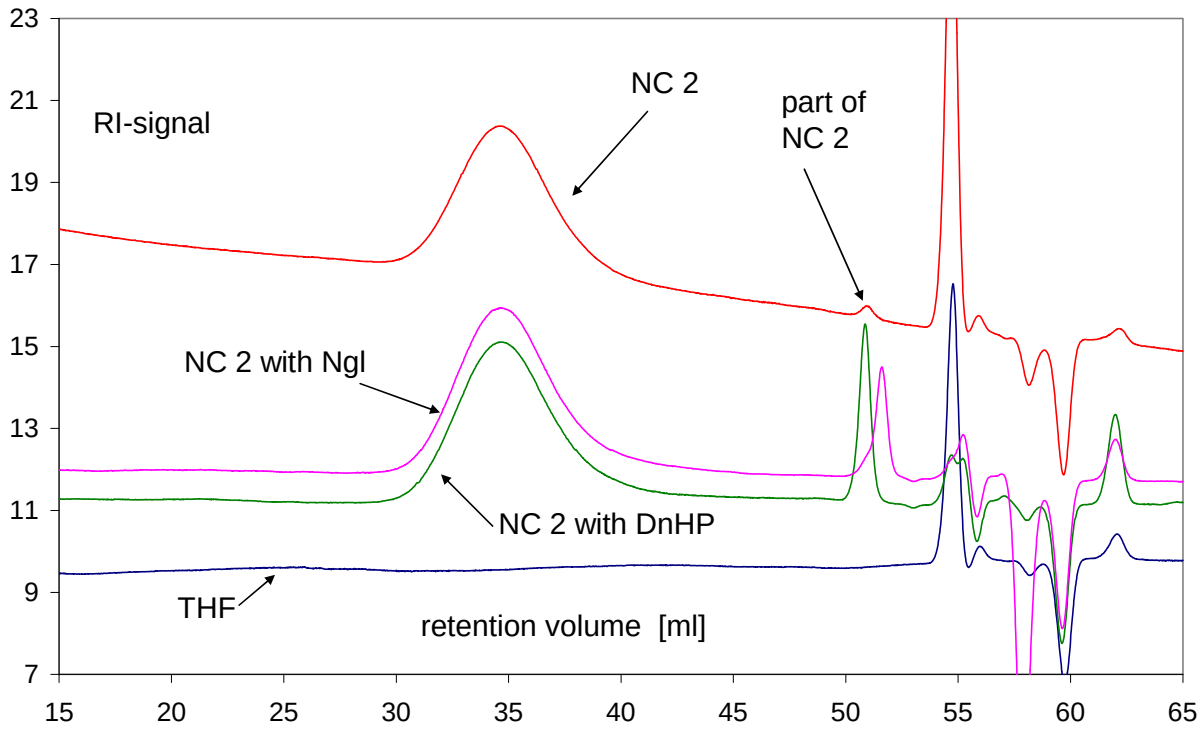

Figure 13: Ngl and DnHP as internal standard with NC 2. Both appear in the same range as the small nitrocellulose peak.

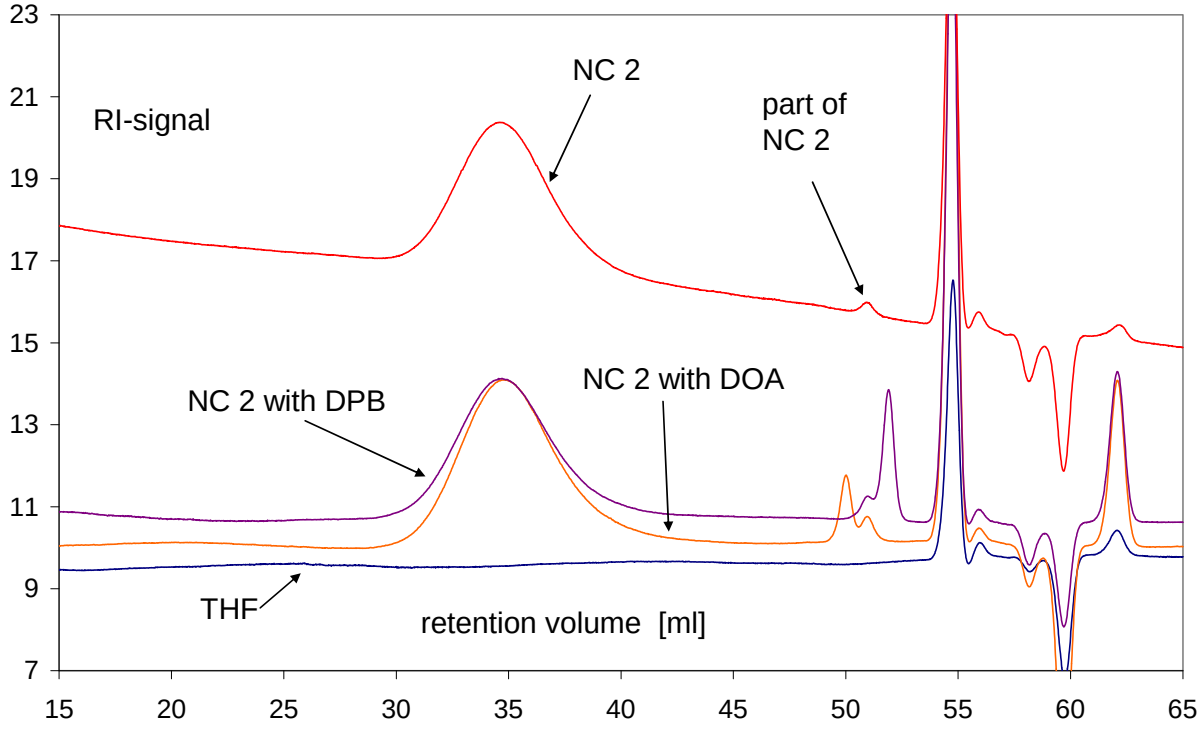

Figure 14: DOA and DBP as internal standards with NC 2. Both overlap with the small peak part of the NC.

4.2.2 Di-octyl adipate (DOA) and di-(n-butyl) phthalate (DBP)

Both Di-octyl adipate (DOA) and di-(n-butyl) phthalate (DBP) overlap with the small peak part of the NC, Figure 14.

4.2.3 Di-octyl sebacate (DOS) and diphenylamine (DPA)

Di-octyl sebacate (DOS) has a distinct peak before the smaller peak part of the NC sample. Diphenylamine (DPA) shows a high peak shortly before the system peak appears. Both of them could be used as internal standards but with DOS the smaller peak part of the NC can not be evaluated together with the main peak of the NC. Figure 15 shows the elugrams of NC 2 with DOS and NC 2 with DPA in comparison with the THF elugramme.

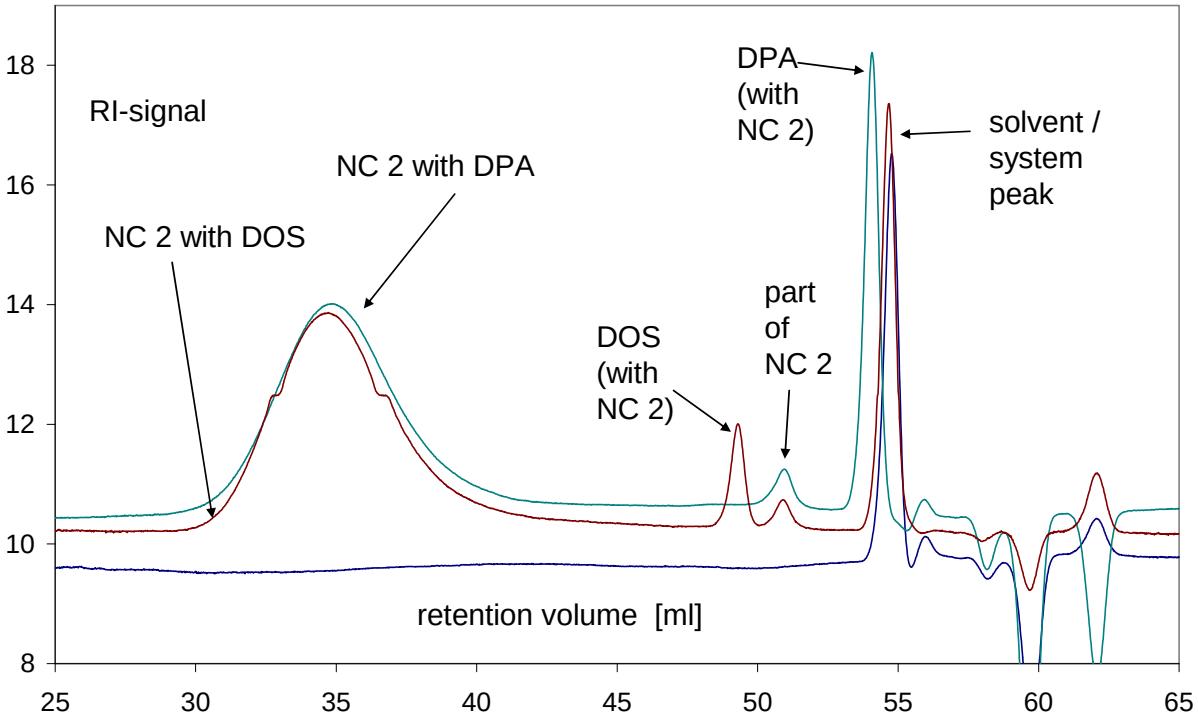

Figure 15: DOS and DPA as internal standards with NC 2.

4.2.6 Conclusion on appropriate substances as internal standards with column set 2: five columns in series with pore sizes of 50, 100, 10^3, 10^5, 10^6 Å

The small peak appearing with NC 1 and NC 2 makes the choice of an appropriate internal standard difficult since some of the possible substances overlap with this low molecular mass part of the NC. If this peak must be evaluated as part of the NC, only di-octyl sebacate (DOS) and diphenylamine (DPA) are usable, while DPA is the better choice since the peak appears with high intensity in the RI-

detector and eluates just before the system peak. The separation of NC with a set of five columns gives a broader eluation range and therefore a more detailed separation. But as disadvantage in using the set with five column could be seen that the already small signal of NC in the refractive index (RI) detector became even smaller.

5. Summary and conclusion

The investigation revealed that it is not trivial to find an appropriate internal standard for the use with nitrocellulose. With column set 1 (two Plgel MIXED-B columns in series) toluene and Ngl failed as internal standards because they were dislocated in the presence of NC. Acardite II tends to overlap with the system peak. But with column sets with a higher separating capacity in the range of low molecular masses the use of Acardite II could be possible. With set 1 two substances revealed to be suitable internal standards: TNT and DnHP. But in both cases the small peak appearing with NC 1 and NC 2 in their low molar mass region must be neglected.

The shift in retention time for toluene and Ngl being in contact with nitrocellulose could be repeated with column set 2 (five single bed columns in series) showing that the behaviour is independent from the columns. Since a small peak belonging to NC 1 and NC 2 overlaps with the signals of DnHP, DPB and DOA, these substances cannot be used as internal standards, if this small peak – representing NC molecules with small molecular mass or an additive – shall be included in the evaluation. Then only DOS and DPA are appropriate internal standards, whereby DPA showing a distinctive signal in the refractive index (RI) detector.

The benefit of the set with five columns is its better separating capacity but it shows a loss in signal height in the refractive index (RI) detector. To allow a detailed separating with an acceptable refractive index signal another combination of columns could be tried, for example 10^6, 10^4, 10^2 Å in series.

6. Literature

[1] Heike Pontius, Manuela Dörich, Manfred A. Bohn,
Gel permeation chromatography of nitrocellulose (NC) and NC containing substances, III. Recent results from a continued parameter study on the GPC of NC samples.
40th Int. Annual Conference of ICT on 'Energetic Materials – Characterization, modelling, validation', June 23 to 26, 2009, Karlsruhe, Germany. Pages 80-1 to 80-12.