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Petroleum Size Exclusion Chromatography. Mechanisms Explaining the NMP front peak

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ABSTRACT

Size Exclusion Chromatography (SEC) is abundantly used in analysis and fractionation of petroleum fractions despite numerous challenges with the techniques. Changing the eluent type amongst classical solvents in SEC normally leads to smaller changes in the elution profile of asphaltenes. Changing the eluent to N-methyl Pyrrolidone (NMP) however leads to very different chromatographic profiles. Especially the occurrence of a very early eluting peak has been severely debated in the literature without reaching a clear conclusion. NMP has particular solvent properties which target the solubility of aromatic molecules and therefore asphaltenes are only partially soluble. In order to understand the occurrence of the front peak in NMP a systematic study is undertaken by changing eluent solvents and temperature. We discuss the different mechanisms involved in SEC such as phase behavior, gel swelling, pore size, molecular size and unwanted adsorption that are all involved in the chromatographic process. To investigate the phase behavior and effects of reduced solubility the elution in mixtures of heptane and toluene is presented. This leads to an increase adsorption but no indication of larger sized aggregates as could have been

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4 expected. Analysis of the difference between NMP, THF and Toluene profiles, indicate that gel
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6 swelling and void volume variation are relatively insignificant and cannot explain the early peak.
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8 This therefore points at a different mechanism based on fluid dynamic effects known from
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10 hydrodynamic chromatography (HDC) of particles, where larger particles travel faster than the
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12 average fluid velocity in laminar flow and arrive at the detector before the average void volume.
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14 Based on the observations on petroleum systems in NMP we therefore hypothesis that HDC is the
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16 driver behind the appearance of the peak in NMP. The insights revealed by the present study show
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18 that SEC can be used also to understand changes in solubility in the eluent solvent e.g. caused by
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20 alteration in chemistry.
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25 INTRODUCTION

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28 The results of Size Exclusion Chromatography (SEC) (also known as Gel Permeation
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30 Chromatography or GPC) which in theory should separate molecules according to size have in the
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32 analysis of petroleum fractions been heavily debated and disputed due to overriding effects not
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34 related to size alone ¹⁻¹⁰. Hence quantitative data in terms of molecular size distributions and
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36 absolute values is often perceived as prone to large errors. The technique is affected especially by
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38 the solute-solvent interactions impacting the solute-stationary phase adsorptive interaction. This
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40 leads to a shift to longer retention and hence lower molecular weights ¹¹⁻¹⁴. There has however been
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42 numerous successful application especially where the aim is not absolute molecular weight
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44 distributions but a relative comparison to understand e.g. asphaltenic deposits, asphalt product
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46 development or refinery conversion processes ¹⁵⁻¹⁹. The association of molecules in poor solvents
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48 could be hypothesized to lead to early elution giving too high MWs.
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Figure 1. Size exclusion chromatogram of asphaltenes in toluene, showing the void volume (time) and the tailing due to non-size adsorptive effects.

Three regions are defined in the chromatogram; void, permeation and non-size. Figure 1. The latter being mainly the adsorption effects while void is the so-called total exclusion. Most often for heavy fractions the total excluded volume (as determined by polystyrene standards) is not reached for the front of the material for ordinary solvents such as THF and Toluene, while a significant tailing is seen beyond a retention corresponding to a molecular weight of zero at the end of the elution. When applying the solvent NMP (N-methyl pyrrolidone) a peak may be observed eluting well before the total excluded volume expected from elution in toluene or classical calibration with polymer standards⁵. A change which has been difficult to relate to changes to the state of the column material.

Herein we attempt to understand and incorporate the different phenomena that affects the chromatographic profile in the interpretation of SEC profiles and especially how NMP eluents results in such different profiles.

First and foremost it is important to keep in mind that “size” in the present context should be seen as a relative term that can cover molecular weight, hydrodynamic volume, or any other size related property that may also be condition dependent. These conditions are:

- Porous stationary phase properties (type, mechanical swelling, interaction with solutes etc.).
- Solvent/eluent power
- Sample, sample concentration and phase behavior
- Change in conformation and aggregation of solutes
- Temperature
- Pressure
- Flow and shearing

In the following introduction we will cover a number of the important mechanisms of retention that affects the SEC or GPC performance as these make the basis for the evaluation of the subsequently presented experimental data on the analysis of asphaltenes and the effect of eluent solvent power.

Most often for heavy petroleum fractions the total excluded or void volume, below or before which no retention occurs, is almost reached for ordinary solvents such as THF and toluene, with a significant tailing beyond a retention corresponding to a molecular weight of zero. When applying the solvent NMP (N-methyl pyrrolidone) a peak at times is observed eluting before the total excluded volume measured in toluene eluent. Figure 2. The prevailing mechanisms cannot explain the occurrence of these early peaks, which were also the center of much discussions in the literature regarding especially analysis of asphaltenes using the SEC technique. (1-4).

It is especially important to investigate this as we recently found that there is a link between asphaltene stability, chemistry and the appearance of the large front peak in N-methylpyrrolidone solvent²⁰. These peaks are the main focus of the present paper, in which we seek to understand how they appear based on a detailed analysis of all common aspects of retention in the chromatographic system.

Figure 2. The appearance of the “front peak” in NMP compared to classical eluents such as toluene or THF. Chromatograms of an n-heptane asphaltenes. Based on ref. 20.

Ideally Size Exclusion Chromatography separates a multicomponent sample based on “size” as the sample flows through a packed bed of porous beads. These monodisperse uniform sized beads are commonly made up from polystyrene-divinylbenzene copolymers that form a network giving high mechanical stability. The process enabled can basically be exemplified as follows under ideal conditions: If a sample consists of three species of very large size (1), intermediate size (2) and small size (3) these will exit the column in that order. Large molecules (if larger than the biggest

pore mouth) will not enter the beads and will exit first at the column outlet, while as size gets smaller the molecules will diffuse and penetrate deeper and deeper into the pack. Under ideal conditions any molecule larger than the pore mouth of the beads will exit at the same eluent volume which is the void or interstitial volume. This is the volume making up the space outside the beads. At the other end of the scale when a very small molecule of similar size as the eluent solvent molecules exits the column this is the total permeation volume as this molecule on average will be able to access all the available pore space. The ideal situation requires that there is no physical interaction between the solute molecules and the surface of the bead material, but this is rarely the case. In the analysis of complex molecules so-called non-size effects may add to the retention mechanism¹². These are effects caused by interactions of solutes and the stationary phase or bead surface through adsorption or specific interactions. As the retention on surfaces is linked to the accessible surface area smaller molecules will be retained more efficiently by non-size effects as they penetrate deeper into the stationary phase, while large molecules have less available surface area on the column material. Hence these effects will lead to a process where the elution is a combination of the size effect and the adsorption effect, the latter being dependent on the nature of the solute. In ordinary adsorption chromatography such as reversed phase chromatography of homologous series of molecules, e.g. n-alkanes, small molecules are retained less than large molecules and therefore small molecules leave the column before large molecules as the same surface area of the stationary phase is available to all species²¹. This could therefore counter balance the adverse effect of the adsorption such that this never completely dominates over the size effect. Figure 3 provides an overview of some of the various mechanisms in the SEC column retention.

Figure 3. Schematics of various mechanisms and variables in Size Exclusion Chromatography.

It is important to select the right eluent for a given type of solute or sample to diminish the non-size effect. The latter may be due to either simple London dispersion forces or specific polar interactions between the benzene rings of the stationary phase and a hydrogen donor such as an alcohol or carboxylic acid group. Early works by Adams and Bicking²¹ details this by analyzing different eluents and the effect of temperature on the elution showing marked effects even for relatively simple molecules. Furthermore the stationary phase will swell differently in different solvents affecting the void volume of the column (see details below). In the analysis of petroleum fractions there is a significant effect of solvent especially when moving from the common solvents such as THF or toluene to solvent with specific interactions such as N-methyl pyrrolidone (NMP), as illustrated in Figure 2. However, NMP has some very attractive advantages as eluent such as apparently reduced non-size adsorption related effects. NMP has therefore been extensively used in coal sciences where the samples are predominantly aromatic in nature ²³ .

SEC in the analysis of petroleum material has for decades been surrounded by controversy due to the non-size effects and lack of proper calibration standards¹⁰ . Numerous developments of calibrations have been presented including the use of discrete petroleum cuts with molecular weight determined by other techniques such as vapor pressure osmometry²⁴ . Often a pragmatic decision is

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4 taken and the molecular weight distribution or average molecular weight is reported relative to
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6 polystyrene standards e.g. ref. 18. – and often times it is acknowledged that the results are not exact
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8 numbers. Hence quantitative data in terms of molecular size distributions and absolute values is
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10 often perceived as prone to large errors. Therefore some researchers have discarded the method as
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12 literally worthless³. Despite of this and in many cases the use of SEC has successfully provide
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14 significant insight into changes and differences in materials as regularly reported in the literature<sup>15-
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16 19</sup>. E.g. Bartholdy et al.¹⁷ reported the change in size distribution of asphaltenes after hydrotreating
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18 of heavy residue using a host of different MW related methods MALDI, VPO, and SEC. All
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20 methods were in qualitative agreement regarding the changes in asphaltene composition as a
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22 function of process conditions. The trend in alteration of these samples were confirmed by Buch et
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24 al.²⁵ using fluorescence depolarization measurements. Therefore we concluded that the SEC or GPC
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26 method as such can provide both cheap and fast qualitative and quantitative relative information
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28 that is useful in process development. It is also important at this point to remember that the property
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30 observed is not size but hydrodynamic volume relate to conformation and it is well-known that even
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32 simple polymers will show differences in hydrodynamic volume dependent on conditions such as
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34 solvent and temperature.
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43 In polymer sciences the hydrodynamic volume is often approximated by the product of molecular
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45 weight and intrinsic viscosity $MW[\eta]$. The widely used semi-empirical correlation that relates
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47 elution volume V_e to $MW[\eta]$ is
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$$\ln MW[\eta] = a + bV_e \quad [1]$$

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55 this is often simply written as
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$$\ln MW = a' + b'V_e \quad [2]$$

Where a , a' , b and b' are calibration constants related to column and equipment parameters. When V_e is replaced with elution time t_e constants obviously change but proportional to the flow rate. However, the calibration may change with eluent solvent power as the conformation and therefore hydrodynamic volume of commonly used polystyrene (PS) standards may change significantly from one solvent to another. The correlation between MW and hydrodynamic volume (R_H) of PS is given by

$$R_H = C \cdot MW^D \quad [3]$$

Where (C,D) are (0.0106, 0.575) and (0.0144, 0.561) for respectively toluene and THF²⁶. These constants seem quite similar but the hydrodynamic volume of PS is about 30 % larger in THF compared to toluene. However, in terms of V_e the observed difference is much less due to various compensation effects.

The elution volume V_e can be expressed as a summation series of contributions from different physical volumes related to the *accessible porosity*. The total active porosity (ϵ_{total}) is made up from the void volume porosity (ϵ_o) - also known as interstitial porosity - and the internal particle porosity (ϵ_p). The latter being the “active” part in size exclusion:

$$\epsilon_{total} = \epsilon_o + \epsilon_p \quad [4]$$

The total physical volume of the column and tubing from inlet to detector can be expressed as follows:

$$V_{total} = V_{void} + V_{pore} + V_{gel(non-pore)} \quad [5]$$

Where V_{void} accounts for the external volume, V_{pore} is the for the eluent total accessible pore volume inside the particles, and $V_{gel(non-pore)}$ is the physical volume of the solvent swelled bead material excluding the pore volume.

As the elution mechanism is based on partitioning between the solvent phase and the internal accessible pore volume the retention volume can for the pure size determining mechanism be expressed as:

$$V_e = V_{void} + K_d V_{pore} \quad [6]$$

Where K_d is a partition coefficient related to the accessible pore volume.

What we will try herein is to expand this series of additive volume contributions to explain and quantify each of them making it possible to better appreciate and utilize the information gathered in experiments.

Adsorption. The solute-stationary phase adsorption is a well known non-size effect which has been extensively investigated by Lafleur et al.¹¹⁻¹⁴. This is one of the commonly mentioned drawbacks in the determination of molecular weights by SEC. The adsorption will lead to increases in retention times and therefore indicate lower molecular weights. This may extend below the determined total permeation time and therefore give negative contributions to the MW. See Figure 1. The adsorption is expected to follow general adsorption thermodynamics, but will also be affected by the accessible column surface area. Hence larger molecules have a relatively smaller accessible surface area than small molecules penetrating deeper into the stationary phase.

In normal and reversed phase chromatography the solute retention is based on adsorption interaction with the stationary phase. This can be described as:

$$\ln k = \ln \beta - \frac{\Delta G}{RT} = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} + \ln \beta \quad [7]$$

Where k , the chromatographic capacity factor, defined as $(t_R - t_0)/t_0$ while ΔG , ΔH and ΔS are respectively the Gibbs free energy, enthalpy and entropy of adsorption and β is the column constant or phase ratio²¹. t_0 is the dead volume or void volume. Adams and Bicking²² used the same

approach to analyze the adsorption in SEC columns observing zero enthalpic contributions for non-interacting species through analysis of temperature effects.

The retention by adsorption can be described through group contribution by adsorption of individual groups such as CH₂, CH₃, aromatic rings etc. and has been extensively used in the prediction of retention (20 and references herein). Using this approach ΔG can be easily expressed by the summation of individual molecular group contributions. For aromatic molecules and especially polyaromatic ring structures the elution is also affected by the planarity and the overall relative arrangement of the rings.

The SEC technique is also affected by thermodynamics of solute-solvent interactions governing in theory the solute-stationary phase adsorptive interaction. Other solute effects may be self-association related to concentration effects²⁷. Self-association should lead to increased apparent hydrodynamic volume and therefore an earlier elution, but the report by Andersen also indicates a more complex effect of concentration. In that study of concentration effects it was clear that a front peak close to total elution also evolved in toluene with increasing asphaltene concentration, but stopped developing at a certain concentration beyond which the SEC distribution profile obtained was basically independent of concentration. However, peaks never appeared earlier than the observed front eluting at low concentrations. Hence this indicated some increase of the content of associated species with concentration, but also an effect of limiting the growth²⁹. (Also see Supplementary material for concentration effects in toluene Figure S1).

Stationary phase swelling. Solvent nature will also affect the retention through the degree of swelling of the stationary phase material ($V_{\text{gel (non-pore)}}$). The swelling properties of the packing material will lead to both changes in the porosity and volume of the packing particles. The latter will lead to a change in the void volume of the column and this will affect the total exclusion³⁰⁻³¹.

Swelling of a cross-linked polymer when changing solvent can be expressed by the difference in the Flory interaction parameter related to the difference in solubility parameter of solvent and polymer. The smaller the difference the more the gel swells within the physical limits of the polymer network. Therefore V_{gel} is a function of the difference in solubility parameters:

$$V_{gel(no-pore)} = f((\delta_{PS-DVB} - \delta_{eluent})^2) \quad [8]$$

In the present case $\delta(PS-DVB)$ is reported to be in the range of 17.9-18.6 MPa^{1/2} which means that swelling should be decreasing in the order Toluene > THF > NMP based on the Flory interaction parameter (Table 1)³². In other words we would expect the void volume to be largest in NMP and therefore one expects a *later* elution of molecules with a hydrodynamic size larger than the porous dimensions of the stationary phase. In reality the actual internal pore volume changes can only be assessed experimentally through the analysis of series of homologous molecules known to have little adsorption contribution.

NMP has been especially attractive over common solvents such as toluene and THF due to the suppression of adsorption. Hansen et al.²⁷ reported this for different PNAs comparing NMP and THF which is reproduced in Figure 4. As seen the non-size effect is significantly reduced in NMP while the PS-standard calibration curve is moved to shorter retention volume with approximately 0.8 ml. This is contrary to the expectation from the above Flory analysis and may be an effect of an elevated temperature for NMP elution. The slopes of the calibration curves show only minor differences indicating that the permeation volume (or bead porous volume) is less affected by the change in solvent. However, one may conclude that the adsorption decreases while the internal pore volume is almost constant and the void is shifted to somewhat shorter volumes but much less than what is often assumed by the front peak appearing for petroleum fractions in NMP.

Figure 4. Retention of PS standards and selected PAHs in NMP and THF respectively. Data from Hansen et al., 27.

Table 1. Selected properties of the eluent solvents and stationary phase material.

	δ_{HIL}	δ_{d}	δ_{p}	δ_{h}	Dynamic viscosity @20°C	Density @20°C	Dielectric Const.
	Solubility Parameter MPa ^{1/2}				cP	g/cc	
Toluene	18.3	18.0	1.4	2	0.56	0.86	2.4
THF	19.4	16.8	5.7	8	0.46	0.88	7.6
NMP	22.5	18.0	12.3	7.2	1.80	1.03	32.2
PS-DVB Column packing	17.9- 18.6*	-	-	-	-	-	-

*) depending on reference (From Barton, 32).

Solute solubility in the eluent. The selection of solvent as indicated above affects both the volumetric property of the column packing, and the adsorption of solutes to the column but it will also affect the conformation and the aggregation state of the solute being examined. While toluene and THF and even heptane-toluene mixtures with more than 50 vol % toluene may be decent solvents for petroleum asphaltenes the partial dissolution of asphaltenes and petroleum fractions in NMP has been reported^{2,9,33}. NMP has a specificity for aromatic molecules over naphthenic structures. E.g. below 54°C NMP is only partially miscible with n-heptane (32). NMP is a common solvent in extraction of aromatics from petrochemical blends of aromatics and aliphatics³⁵. Ascanius et al.³³ reported extraction yields from asphaltenes using NMP at room temperature in the range of 40 to 90 % while we in unpublished data has found extraction yields as low as 5 %. In SEC analysis often times the petroleum fraction has been dissolved in the NMP and filtered prior to injection into SEC unit to avoid blockage of the column by solid material. This obviously leads to an incomplete analysis of the fraction, although the filtered sample may still contain particles smaller than the nominal pore size of the filters, which often is in the range of 0.2 μm or 200 nm. Mapolelo et al.,²⁰ reported the administering of asphaltenes by injection of toluene solutions into the NMP stream of the HPLC injector with no apparent pressure build up over significant time of operation and more than 100 injections. The injected solutions were 2 g/L but with a 20 μL injection this is only 0.04 mg material and if only contacted by roughly one pore volume this is a concentration of approximately $0.04 \text{ mg}/4 \text{ ml} = 0.010 \text{ mg/ml}$ or 10 ppm. This will also keep the concentration effect constant if we assume the separation has no influence on solution kinetics e.g. association or dissolution within the time of analysis which is approximately 10 minutes.

Through a meticulous analysis of the individual effects mentioned above we will try to explain the occurrence of the front peak in N-methylpyrrolidone eluents. This will be done through analysis of increasingly poorer solvents (heptane-toluene mixtures), analysis in toluene, THF and NMP, and

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effects of temperature. The key point is to determine the development of the three major retention zones: the interstitial void, the permeation zone and finally the overriding non-size effects. This may explain how a major peak can occur in NMP. We will show that the partial solubility of asphaltenes in NMP indeed is the reason for the front peak observed for less stable asphaltenes, and that the front peak is due to formation of particles large enough *to move faster* than the average eluent flow rate therefore emerging before the void volume. The latter mechanism is known from the niche chromatographic science called hydrodynamic capillary chromatography or sometimes for packed beds of non-porous beds referred to as “slalom chromatography”³⁶⁻⁴⁰ .

EXPERIMENTAL

Asphaltenes samples were obtained by n-heptane precipitation following a modified IP 143 method. For studies of temperature effects and the effect of mixed eluents (toluene-heptane with up to 40 % heptane) the petroleum source was Boscan crude oil. For properties of these Boscan asphaltenes see table 2. For the study of effects of change in solvents THF, Toluene, NMP the asphaltenes came from a heavy oil source of unknown original. However, the results have been observed to be consistent over a large body of different samples²⁰. Sample concentrations were 2 g/L. The solutions were sonicated for 30 minutes and left overnight to equilibrate.

Table 2. Selected properties of Boscan asphaltene sample used in heptane-toluene and temperature experiments.

Boscan asphaltenes	
C% w/w	80.6
H/C atomic	1.20
N/C atomic	0.02
S/C atomic	0.032
Asphaltene content in crude wt %	19.2

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4 All solvents were HPLC grade. Mixtures were prepared volumetrically at room temperature and
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6 degassed by boiling and sonication.
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10 Different HPLC-SEC units have been employed. For the study of heptane-toluene and temperature
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12 effects the system consisted of a Gynkotec 300 HPLC pump, a Rheodyne 7725 injector with a 20 μ l
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14 loop, a Jasco 910 DADetector and a Knauer RI detector. The latter was used for the analysis of PS
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16 standards. Linearity of the DAD detectors at relevant wavelength settings was investigated in all
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18 eluent mixtures. For the NMP, Toluene and THF comparison studies either the above system but
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20 fitted with a PE model 235C DAD was used or we used a Jasco PU 2086Plus HPLC unit with a
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22 Jasco MD2015Plus UV detector. Details are also given in Figure 5. As indicated above despite a
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24 time span of many years the use of the same columns and settings provided performance almost
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26 within experimental error.
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31 The main settings for the two series of experiments are indicated on Figure 5. Two different
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33 columns from PLGel, Polymer Laboratories, namely Mixed D and Mixed C were used. These have
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35 somewhat different ranges in terms of total exclusion as indicated in Figure 5. Polystyrene
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37 standards from PolymerLaboratories were used for the calibration of the column performance and
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39 consistency as outlined above in the introduction.
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Figure 5. Columns and settings for the various HPLC-SEC tests.

RESULTS AND DISCUSSION

As the stability of SEC systems has been questioned in the literature^{1,3,4} albeit unsupported by data, we initially want to verify the stability and reproducibility of the often used PLgel columns with a broad MW range application (so-called mixed phase columns). Reported calibration data based on polystyrene standards and from 3 different groups spanning 20 years are plotted in Figure 6 according to Equation 2^{7,20,27}. All used the same conditions namely a PLGel Mixed D column, same column dimensions, NMP as eluent, flow rate 0.50 ml/min and a column temperature of 80°C. As observed all three research groups independently obtained almost identical results. The data also indicates that we can assume the excluded void volume to be between 5 and 6 ml in NMP based on PS elution. Small deviations may be caused by guard columns, different size and length of connection tubings etc. Overall the conclusion is that these columns show remarkable consistency across time and research groups making it possible to compare data across the literature and therefore providing a basis for direct comparison of data. One reference often cited in asphaltene sciences directly explains that results are erroneous as the SEC column material cannot withstand organic solvents³. However, this argument is not founded in reality as most columns are made for

use entirely with organic solvents including common solvents such as toluene, THF and NMP. It could be that the author of that paper misunderstood the known experimental fact that abrupt changes in eluent strength may lead to irreversible damage. It should be clear that data in Figure 6 certainly indicate that SEC do not suffer from stability or reproducibility issues.

Figure 6. Polystyrene standard elution in NMP on PLgel MIXED D column performed by 3 different independent groups over 2 decades showing remarkable consistency. Data from ref 7, 20, 27.

Although the lack of appropriate calibration standards has been the most debated issue in the literature the more important adverse effects, however, are non-size effects which distort the elution order and therefore leads to wrong results e.g. if the profile is used to calculate molecular weight related properties.

As indicated in the introduction the following aims at understanding how the change in solvent from Toluene to NMP can lead to a significant change in the generated “size distribution” and why

in NMP a distinct “front” peak is observed for some samples. And as mentioned above there has been a substantial debate on whether this peak is related to aggregation of molecules or very large molecules eluting faster as the adsorption is diminished by NMP²

We will analyze three different effects in particular which we known could be related to differences between toluene and NMP in terms of solubility behavior or conditions of applications:

- Effects of decreasing solvent power
- Effects of temperature: NMP is always used at high T, while solvents such as toluene or THF regularly are applied slightly above room temperature.
- Variations of column performance induced by eluent-stationary phase interactions.

Decreasing solvent power: In order to understand solvent/eluent effects we evaluate the effects caused by a decreasing solvent power of the eluent towards the asphaltenes. This can be done by increasing the heptane content in toluene eluent without reaching the precipitation onset, and injecting the same concentration of asphaltenes. In theory we would expect more association of the asphaltenes in bulk and also an increased adsorption effect. Increase association would be expected to yield a larger front peak. This approach allows us to stay below the asphaltene onset point or solubility limit as toluene and heptane defines our samples and the onset is known. This is contrary to the case of NMP, where the solubility limit may be highly dependent on the asphaltene chemistry.

Figure 7. Effect of eluent composition (heptane-toluene mixtures) on the SEC profile of Boscan asphaltenes. Expansion of X-axis to 20 ml is reported in Figure S1.

In Figure 7 it is observed that the main effect of the increased heptane content is an increased adsorption to the column, leading to significant tailing in particular as the content is increased beyond 20 vol% heptane in toluene. (Data up to 20 ml is provided in Supporting Material Figure S1). One important observation is that the initial retention time of the chromatogram is only affected slightly but contrary to expectations of increased association it moves to longer retention times. No front peak that could reflect increased association or phase separation in the heptane-toluene mixtures is observed²⁸. By comparison with Figure 2, the conclusion is that a decreasing solubility power is not emulating the NMP behavior as no peaks are observed before approximately 7 minutes. A particular interest should be paid to the peak appearing at about 10.5 ml, which appears to be somewhat independent of the change in eluent. This peak has been related to smaller porphyrins both through use of standards and the well-known Soret absorbance response at 410 nm detector setting. Apparently this particular range of porphyrinic species are not prone to significant adsorption and may therefore serve as an internal standard that can provide information on the changes in solvent-column interactions e.g. changes in void volume. In supporting information

Figures S2 and S3 we report the influence of detector wavelength setting on the SEC profile for two different eluent compositions with retention volumes up to 20 ml. In supporting information we have also included the concentration effect between 2 g/l and 40 g/l for elution in toluene as Figure S4 which although the front elution moved slightly to shorter retention did not show any significant signs of association as previously reported by us for other asphaltenes²⁸.

To further quantify the adsorption the area between 6 and 12 minutes (from total exclusion to the approximated total permeation time for the eluent) was determined assuming a constant absorption coefficient for all species. The area under the chromatogram is significantly reduced as material is adsorbed, elutes later and moves beyond the target retention time range. In the 40 % heptane eluent only about 40 % of the sample is observed in the window of operation, Figure 8. As a control the linearity of the detector was confirmed for all eluent composition by direct injection of dilute solutions of asphaltenes into the detector. This also confirmed that a change in the solvent is not affecting the average UV-vis absorption coefficient significantly for the sample at the applied 2 g/L concentration.. (See data in Supporting Material Figure S5 and Table S1). Further the polystyrene standard of MW 2050 g/mole showed that retention was increased around 1 minute as heptane content increased to 40 vol% either due to a reduction in hydrodynamic volume of the PS or an increased void volume leading to later elution. However, which mechanism prevail could not be determined. Figure 9.

Figures 8. Recovery of eluted material by integration of the UV signal vs eluent composition in the interval from 6 to 12 minutes.

Figure 9. Retention of polystyrene standard 2050 g/mol as a function of heptane/toluene eluent composition.

Temperature effect. In order to use NMP as an eluent the general practice is to increase temperature to decrease the viscosity and thereby the differential pressure over the column. The increased temperature will in most cases lead to a decreased adsorption to the column, and could also lead to differences in column properties as well as various degrees of dissolution states (dissolved vs aggregated asphaltenes).

Figure 10. Effect of temperature on the HPLC-SEC profile of Boscan asphaltenes in toluene eluent.

To capture the temperature effects without introducing the special solubility properties of NMP we examined the elution in toluene of Boscan asphaltenes between 30 and 90°C. As observed in Figure 10 there is a regular decrease in the elution time at the rear end of chromatograms while the front was only changing very little except between 30 and 40°C. The quite significant move to shorter retention times within this interval is surprising but may be a combine effect of several mechanisms. Overall the temperature effect seems to indicate that mainly the adsorption at the back side of the elution profile is reduced as we increase the temperature. To examine this further one may plot the absorbance in the tailing section versus $1/T$. The results in Figure 11 indicate almost a purely enthalpic effect and the enthalpic analysis confirms at least to some extent that the main effect in this part of the chromatogram is due to adsorption changes and that this is reduced due to the increase solubility of the asphaltenes at elevated temperature as substantiated by Andersen⁴¹. However, we also observe that the porphyrin related peak at about 10 minutes change position to

about 9.2 minutes, which would indicate a swelling effect of the stationary phase. There is no indication that dissociation is controlling the outcome of the temperature increase. In that case one would expect an increase in smaller sizes and a reduction of the main peak at about 8 minutes or as frequently speculated an decrease at the front of the profile. In Figure 10 we observe a change in absorbance at 8 minutes between 30 and 50 after which it is reduced again to an almost identical shape of the front profile as the temperature increases further. It is important to recall that the relation between retention time and hydrodynamic volume is logarithmic and hence the analysis could over-emphasize the effect at longer retention and smaller size.

Figure 11. Enthalpic analysis of the retention at 11 minutes (adsorption part of chromatogram (in Figure 10) using the output of the detector as proxy for concentration. Boscan asphaltenes in toluene.

Figure 12. The relative variation of classical contributions to size chromatography in Toluene/Heptane; Toluene & THF and NMP. Accordingly the main effects should be within the “non-size” effects. Position of porphyrin related peak indicated.

Solvent effects on column performance: Based on the analysis of the classical mechanisms it is seen that the main effect of a change in solvent appears to be related to the long term non-size effect. Figure 12. This is related to the adsorption to the stationary phase which leads to variations in the longer retention times as given in Figures 2 and 7. For NMP one could envision that the observed reduction of adsorption and elution beyond the eluent retention point is further reduced by the increased column temperature required to reduce the viscosity effects. The observed change in PS calibration standard elution also indicates that the improvement by NMP might mostly be in the temperature effect. The specific solubility enhancement of aromatics in NMP relative to the other eluents further improves this.

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4 Concluding on the above it is safe to say that no classical mechanisms or combinations of these can
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6 explain a change leading to such a large change in elution leading to a new peak at very short
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8 retention time as abundantly reported for NMP when compared to either THF or Toluene.
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14 **Hydrodynamic effects and flow of particulate matter.** As the above brings no explanation to the
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16 appearance of an early eluting peak outside the normal SEC behavior, we therefor need to search
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18 for another explanation. One often overlooked non-chromatographic mechanism in SEC is the
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20 hydrodynamic effects that may lead to separation due to particle size, also known as hydrodynamic
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22 chromatography (HDC). This mechanism seems to be the only plausible one that explains the
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24 occurrence of the early peak in NMP. In NMP the partial insolubility of asphaltenes may create
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26 finite particles that may form either in the injected solution or when contacting the NMP in the
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28 column^{27,33}. While a decreasing solvent power of the eluent normally leads to increased column
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30 adsorption more dominant than e.g. association, there is a turning point where the occurrence of
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32 micro phases will start to behave as non-interacting particles. These particles will separate
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34 according to hydrodynamics and will travel at a linear velocity higher than the average flow rate
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36 through the column. As total exclusion is generally determined by the average velocity of the eluent
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38 the particles formed will penetrate the column earlier leading to early breakthrough and the
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40 occurrence of a new peak. Nano-aggregates would not behave this way due to the relatively small
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42 size (shown by elution in heptane-toluene blends) and hence in NMP the front peak indicates that
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44 larger particulate matter constituting a separate phase must have been formed. The reduced
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46 adsorption in NMP is due to the strong NMP-aromatics interaction, while the particulate micro
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48 phase matter should be formed by asphaltenes with a lesser aromatic structure. This is in agreement
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50 with the fact that NMP insoluble materials normally have very low fluorescence yield indicating a
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52 low degree of conjugation of double bonds, and therefore low aromaticity^{9,27}. Herein these
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mechanisms will be dealt with in detail concluding that SEC indeed provides very valuable information on the nature of the material analyzed which can be useful in both petroleum geochemistry, flow assurance and processing in refinery operations. Smaller aggregates of molecules would still be considered to move along a classical SEC separation path. In particular the explanation may be found in the science of hydrodynamics of particle flow in beds of non-porous bead beds – or in this case porous beads which the “solute cannot penetrate”.

Apicella et al.⁶ showed that SEC analysis of particulate soots resulted in material eluting before the total exclusion limit as it elute within the void. They also related the observation of a gap between the first peak and the regular elution as an indication of moving from a 2D molecular structure to a 3D structure. This is in agreement with the observation of hydrodynamic chromatography behavior as proposed herein.

The fairly small niche area of hydrodynamic chromatography is used to analyze particles in capillaries, or in packed and gel beds. As the aspect ratio between the channel radius and the particle radius decrease the particles are more and more excluded from the flow close to the tube wall. Hence they travel faster than the average flow. In the simplest theoretical approach the flow behavior in beads and gels is considered to be approximated by laminar flow in a bundle of capillaries with average radius of R . To simplify the behavior it is assumed that there is no flow inside the porous beads where transport takes place mainly through diffusion. The separation of particulate matter into sizes takes place through the following simplified mechanism: For Poiseuille flow the linear flow rate is highest in the middle of the capillary tube as shown in Figure 13 and this is where the biggest particles will flow while smaller particles will flow slower and closer to the wall³⁷⁻³⁸. Stegeman et al.³⁷ already in 1991 commented that combined HDC and SEC effects were commonly seen with modern SEC columns and that it leads to misinterpretation/calibration of the

log MW relations as the inter-particle volume becomes difficult to determine. This is especially so as it becomes difficult to assess the actual void or interstitial volume.

Figure 13. Flow of particles in tube. The center of mass of the larger particles are excluded from the low velocity regions near the tube wall, resulting in a higher velocity than the average Poiseuille flow.

The analysis is made relative to the void volume and HDC happens in the range where $V_i < V_o$, and therefore the relative retention $R_F = V_o/V_i$ is larger than 1 contrary to ordinary chromatography.

This is also why a particle with radius r_p and larger than the pore mouth of the porous bead, will leave the column at or even before the average void flow. Tijssen and coworkers have reported the relation between the relative retention R_F and the radius of the assumed average capillary R and the particle r_p to be expressed as⁴²:

$$R_F = \frac{V_o}{V_i} = 1 + 2\frac{r_p}{R} - C\left(\frac{r_p}{R}\right)^2 \approx 1 + 2\frac{r_p}{R} \quad [10]$$

C is a constant which for simple Poiseuille flow is unity, but it has been reported to be as high as 5.335). However, for rather small value of r_p/R the third term might be neglected. As we here only try to explain the phenomena observed we will assume this to be correct. In order for HDC effects to become noticeable Striegel and Brewer³⁹ reported that the aspect ratio r_p/R should be larger than 0.02. With a particle size of 5 μm the lower limit is in the range of 0.1 μm which is plausible for asphaltene particles.

R the assumed hydraulic radius of the gel is derived from the packing particle size d_p and the porosity ε .

$$R = \frac{d_p \varepsilon}{3(1 - \varepsilon)} \quad [11]$$

By using these approximations and the observed difference between the approximate V_o from Figure 4 and the peak appearing in NMP (Figure 2) we can estimate the particle size needed to produce the front peak. Average porosities of PS-VB have been reported to be in the range of 0.43-0.52, and commonly for packed columns the porosity is often mentioned to be 0.39. In this case using the column dimensions the void volume ε is approximately 0.49-0.52. Hence the estimated hydraulic radius is about 1.6 μm .

The elution volume of the front peak in NMP is approximately 3.4 minutes while the void volume from the PS analysis (Figure 4) is about 5.5-6 minutes giving a R_F value of approximately 1.6-1.7. By applying these magnitudes we can make a rough estimate of the average radius of particles leaving the column at 3.4 minutes to be in the range of 0.40-0.60 μm . This is 2 orders of magnitude larger than reported estimated values of the radius of nano-aggregates of asphaltenes. As the only mechanisms that can generate such large particles will be as phase separation and/or flocculation we must conclude that the most likely reason behind the appearance of the front peak is that certain NMP insoluble asphaltenes aggregate and form large particles even at very low concentrations. Given the specificity of NMP towards aromatic substances the phase separating material are potentially alkyl or naphthenic rich molecules that flow through the void volume without sticking. This is supported by the fact that apparently alkyl rich low fluorescent asphaltenes has a higher propensity to exhibit the front peak when eluted by NMP-SEC^{20,33}. Other reports on coal products show no front peak indirectly supporting this as coal is very rich in aromatic moieties and hence coal products will be preferentially dissolved in NMP and no second phase is formed².

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4 In conclusion the retention of asphaltenes or other petroleum products in HPLC-SEC and in
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6 particular when using NMP as eluent must be described as a combination of both hydrodynamic
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8 and chromatographic effects. Obviously this also means that the appearance of the front peak
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10 should not be accounted as part of a molecular size distribution. However, on the other hand the
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12 remaining later eluting part of the chromatogram could potentially show true features of the NMP
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14 soluble material especially due to the suppression of the column adsorption.
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18 **Shearing effects.** Streigel⁴³ has shown that changes in flow may affect the conformation of
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20 dissolved polymers standards. However, in the flow regime applied herein 1.0 and 0.5 ml/min the
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22 change was not significant compared to the above reported effect of solvent change. Also the
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24 reported effect was an obvious increase in retention time with increasing flow and therefore this
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26 cannot add evidence to the occurrence of the peaks in NMP.
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30 CONCLUSION.

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33 In the above analysis we have been able to reject a number of concerns about the applicability of
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35 size exclusion and gel permeation chromatography to the analysis of petroleum derived materials.
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37 While the molecular weight issue remains and may be difficult to resolve due to chemical
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39 complexity, it is clear that many of the reported and alleged deficiencies of SEC have been ill-
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41 founded and biased with no root in the actual science. Through a thorough analysis of known
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43 individual contributions and mechanisms we find that:
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48 1) there is no substantial deterioration of the column material by solvents such as toluene, THF or
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50 NMP as otherwise stated in ref. 1 and 3.
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53 2) calibrations can be repeated between laboratories when the same column material, eluent, and
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55 temperature is used showing that columns are both stable and reproduced in the above organic
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57 solvents.
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3) fundamental sciences such as adsorption, diffusion and fluid dynamics can basically explain all the observations even when there is a substantial difference in chromatograms such as in the case of a change from toluene to NMP.

4) the early peak appearing in SEC analysis of asphaltenes and other petroleum products when using NMP as eluent is apparently due to local phase separation leading to hydrodynamic chromatography (HDC) where particles travel faster than the average flow rate. The particle formation we suggest is due to the specific selectivity of NMP which leads to local phase separation of alkyl or naphthenic rich asphaltene molecules.

5) When using NMP as eluent in SEC of petroleum products it is therefore important to understand that results are a combination of SEC and HDC. This fact was never included in the previous discussions of the origin of the front peak in NMP, and we hope this paper resolves the matter.

ASSOCIATED CONTENTS

Supporting Information is available free of charge XXXXX

Figures S1-S5 and Table S1.

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Notes

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