

## Recommendation of RILEM TC 295-FBB

### Implementing a systematic approach to fingerprint bituminous binders using proton nuclear magnetic resonance spectroscopy ( $^1\text{H-NMR}$ )

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# Recommendation of RILEM TC 295-FBB: Implementing a systematic approach to fingerprint bituminous binders using proton nuclear magnetic resonance spectroscopy ( $^1\text{H-NMR}$ )

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**Abstract** This recommendation is an output of a small-scale round-robin test involving three different laboratories from TG2 of the RILEM TC 295-FBB: “Fingerprinting bituminous binders using physicochemical analysis” concerning the use of  $^1\text{H-NMR}$  for fingerprinting of bituminous binders. It demonstrates the full capabilities of  $^1\text{H-NMR}$  as a robust characterisation tool for complex organic materials,

like bituminous binders, to examine their molecular composition in a reproducible way and with the best possible detail. This recommendation documents the key factors in sample preparation and the sensitivity of data post-processing steps. It concludes with best practices and a case study examining the effect of laboratory ageing on two bituminous binders. Overall, it highlights the potential, to the broader scientific

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community, of another efficient chemometric tool for bituminous binders.

**Keywords** Bituminous binder · NMR spectroscopy · Molecular fingerprinting · Reproducibility

## 1 Introduction

Bituminous or asphalt binders are the cornerstone of road pavements. They offer excellent viscoelastic properties, have superb waterproofing behaviour and provide sufficient binding interactions with the rest of the mixture components, namely mineral aggregates and filler [1]. As such, bituminous binders have long been studied from a material perspective to understand their intrinsic characteristics and their effect on mechanical performance. A new era has begun in asphalt research under the generic umbrella of chemomechanics of asphalt binders, which intends to provide a link between chemical composition and performance.

Undeniably, the study of the mechanical performance of binders is of paramount importance for pavement design, and numerous research works have been devoted to this research line. However, the chemical characterisation of bituminous binders is still an area without complete consensus, often due to the lack of standardisation protocols that could guarantee a systematic sample preparation and testing as well as comprehensive data processing [2]. In this regard, efforts to study the chemical composition at the molecular level have been focused primarily on Fourier Transformation Infrared (FTIR) spectroscopy studies, for which previous task groups (TGs) of the RILEM Technical Committees (TCs), as well as current TG1 of the TC 295-FBB, have been dedicated [3–5]. Other robust techniques for chemical

fingerprinting do exist but are rather underutilised, while challenges arise in their systematic evaluation for bituminous binders. The TG2 of TC 295-FBB aims to address these challenges via a small-scale round-robin and overcome common fallacies during sample preparation, devices and data processing. The primary focus is on the utilisation of Proton Nuclear Magnetic Resonance ( $^1\text{H-NMR}$ ) to gain deeper insights into the chemical composition of bituminous binders with the potential of improving and strengthening our understanding on a fundamental basis. This could assist later in systematically intervening into the material's fundamentals, i.e. via binder modification, to offer eventually a superior mechanical performance.

NMR is a spectroscopic technique invented in the 1940s, with original applications in condensed matter physics [6]. The fundamental concepts of NMR spectroscopy, while rooted in quantum mechanics, can often be described (at least approximately) using classical analogies that are accessible and intuitive. One such analogy involves nuclear spin. Certain nuclei, specifically those with an odd number of nucleons, possess a property known as nuclear spin. Nuclei with spin exhibit a magnetic moment, allowing them to interact with an external magnetic field by aligning either with or against it. When subjected to radiofrequency radiation, these spin states can be excited, resulting in transitions between them. The energy required for these transitions is highly sensitive to the chemical environment surrounding the nucleus. Consequently, hydrogen atoms in aliphatic compounds can be distinguished from those in aromatic compounds using this technique. To obtain chemical information that is independent of the device used, the concept of chemical shifts was introduced. The chemical shift of a specific proton  $H_i$  is defined in Eq. 1:

$$\delta_{H_i} = \frac{\nu_{H_i} - \nu_{\text{Reference}}}{\nu_{\text{Spectrometer}}} * 10^6 \quad (1)$$

$\delta_{H_i}$ ...Chemical shift of proton  $H_i$  (ppm).

$\nu_{H_i}$ ...Resonance frequency of proton  $H_i$  (MHz).

$\nu_{\text{Reference}}$ ...Resonance frequency of the protons in a reference standard (e.g. tetramethylsilane) (MHz).

$\nu_{\text{Spectrometer}}$ ...Spectrometer frequency (MHz).

The  $^1\text{H-NMR}$  spectrum is a graphical representation that depicts the NMR intensity (which is directly

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correlated to the abundance of specific molecular groups) against the chemical shift. This spectrum is unique for different molecules and macroscopic materials such as bituminous binders. It is apparent that since bituminous binders consist of a vast number of molecules, the resulting NMR spectra are often challenging to interpret.

So far, the use of NMR spectroscopy to study fundamental changes of bituminous binders after laboratory ageing or due to environmental conditions in the field has been very limited [7–13]. The overlapping chemical shifts make the overall evaluation of an  $^1\text{H}$ -NMR spectrum a challenging task, while showing the sensitivity of data post-processing as the changes are often minor. Limited studies explored the effect of the solubility-based SARA fractions of bituminous binders, reporting an increase of aromatic regions within the most polar fractions [14–16]. A combination of different NMR techniques in crude oils shows promising results in unravelling the origin or refinery process of the crude [17–19]. However, it has only recently been utilised in bituminous applications combining more advanced NMR techniques, i.e. using  $^{31}\text{P}$  and proton-carbon single bond correlations, to shed light into detailed chemical information of the bituminous binders' composition [20, 21].

Despite the increasing interest over the last decade in elucidating the chemical composition of bituminous binders using more robust techniques like  $^1\text{H}$ -NMR, the influence of sample preparation and the device operational settings on the obtained results remains unexplored in the current state-of-the-art. It is crucial that efforts towards a commonly accepted sample preparation procedure are undertaken by scholars worldwide who are willing to comprehensively fingerprint the bituminous binders' structure, i.e. to study differences based on the crude source or refinery and due to different ageing states. Hence, the  $^1\text{H}$ -NMR data processing becomes another important factor of consideration for complex materials like bituminous binders as compared to pure samples that are commonly used in routine  $^1\text{H}$ -NMR.

## 2 Objective

This recommendation of TG2 of TC 295-FBB marks the first reported work for a systematic evaluation of reproducibility and examination of

the sensitivity of data-processing for  $^1\text{H}$ -NMR of bituminous binders. While some of the principles adopted are well-known and widely applied in other fields of materials characterisation, this work adapts them to address the specific challenges of the  $^1\text{H}$ -NMR analysis at the bituminous binder level. The main objectives of the recommendations proposed in this work are as follows:

- To analyse the reproducibility of  $^1\text{H}$ -NMR measurements for bituminous binders accounting for both the sample preparation and device settings.
- To quantify the statistical error associated with each data processing step in  $^1\text{H}$ -NMR analysis of bituminous binders.
- To apply the best practices in a case study within a small-scale RILEM round-robin test to examine the effects of short- and long-term laboratory ageing on  $^1\text{H}$ -NMR results.

### 2.1 Participating laboratories

The participating laboratories of TG2 295-FBB, alongside their abbreviation, are given in Table 1. It is noteworthy that each laboratory performed the  $^1\text{H}$ -NMR in their own facilities, following their in-house sample preparation for the same bituminous samples, while the data processing was performed upon agreement with the TG by one participating laboratory. It should be noted that the different bituminous binder samples were all conditioned and prepared in a single laboratory (i.e. TU Wien), which were later distributed to the other participating laboratories for further processing and preparing the respective  $^1\text{H}$ -NMR samples.

**Table 1** Participating laboratories in the RILEM TC 295-FBB TG2 reproducibility study

Laboratory name	Country
TU Wien	Austria
University of Antwerp	Belgium
University of Waterloo	Canada



### 3 Materials and methods

#### 3.1 Materials

The materials used for this study have been selected for the purposes of the entire TC 295-FBB based on two criteria: similarity of penetration grade and variability in supplier and crude oil origin. To assess whether binders with comparable empirical properties differ in their chemical fingerprinting as revealed by  $^1\text{H-NMR}$ , samples were chosen that fall in the same penetration classification according to EN 12591. The empirical properties confirming this similarity are summarized in Table 2. At the same time, the binders were sourced from different suppliers, potentially reflecting variations in crude origin and refinery process. This criterion allows for an evaluation of binder variability at molecular level.

#### 3.2 Laboratory ageing protocols

Laboratory short-term and long-term ageing of both binders was conducted at a single laboratory at TU

**Table 2** Empirical properties of the unaged bitumen samples

Sample code	Needle penetration (0.1 mm)	Softening point ( $^{\circ}\text{C}$ )	Penetration index ( $I_p$ ) (-)
B1198A	85	47.0	-0.66
B1199A	84	45.8	-1.05

**Table 3** Device, acquisition parameters, solvent and mass concentration

Parameter	Laboratory I	Laboratory II	Laboratory III
Device	Bruker Avance II 400 MHz	Bruker Avance III HD 400 MHz	Bruker Avance II 300 MHz
Resonance frequencies	400.13	400.13	300.13
Delay time (s)	1	1	1
Size of FID	32,768	65,536	16,384
Number of Scans	64	32	16
Acquisition time (s)	2.27	4.09	1.89
Spectral width (ppm)	18.02	20.03	14.46
Average S/N ratio	25,000	14,000	1000
Solvent	Deuterated chloroform ( $\text{CDCl}_3$ )	Deuterated tetrachloroethane ( $\text{C}_2\text{D}_2\text{Cl}_4$ )	Deuterated chloroform ( $\text{CDCl}_3$ )
Average sample concentration (mg/ml)	33.34	30.77	20.54

Wien. The Rolling Thin Film Oven Test (RTFOT) method, according to EN 12607–1, was used to simulate short-term ageing at 163  $^{\circ}\text{C}$  for 75 min. Long-term ageing was simulated by using the Pressure Ageing Vessel (PAV) method according to EN 14769 at 100  $^{\circ}\text{C}$  and 2.1 MPa with an ageing duration of 20 h. All samples were homogenised, flushed with nitrogen, and sealed before shipping. Unaged, RTFOT-aged, and RTFOT + PAV-aged samples are referred to as A, B, and C throughout this manuscript. In total, each participant in this study received six binders:

B1198A (unaged), B1198B (RTFOT), B1198C (RTFOT + PAV)  
 B1199A (unaged), B1199B (RTFOT), B1199C (RTFOT + PAV)

#### 3.3 Proton nuclear magnetic resonance ( $^1\text{H-NMR}$ )

Proton ( $^1\text{H}$ ) NMR spectra were measured for each sample twice. For that, ~20 mg of each sample was dissolved in 600–650  $\mu\text{l}$  deuterated solvent, giving a similar concentration for all the laboratories during the measurement. This reduces variations due to the impact of bitumen solubility within the chosen solvents. The exact acquisition parameters, devices used, solvents and employed sample concentrations are shown in Table 3.

Preliminary measurements at one of the three laboratories investigated the effect of delay time on the quantification of NMR data from bitumen, which was evaluated by employing delay times of 1, 10, 20, and



30 s on both binders in the unaged state. The raw data evaluation routine of the obtained NMR dataset was implemented by one laboratory. The importance of each processing step was assessed by varying each parameter and evaluating the average deviations/errors between the laboratories. The parameters that were varied during the evaluation procedures were phasing, baseline correction, signal-to-noise ratio, and calibration methodology. Their expected impact on the quantification is discussed in detail in the respective sections.

### 3.4 Statistical methods

#### 3.4.1 Kruskal–Wallis test

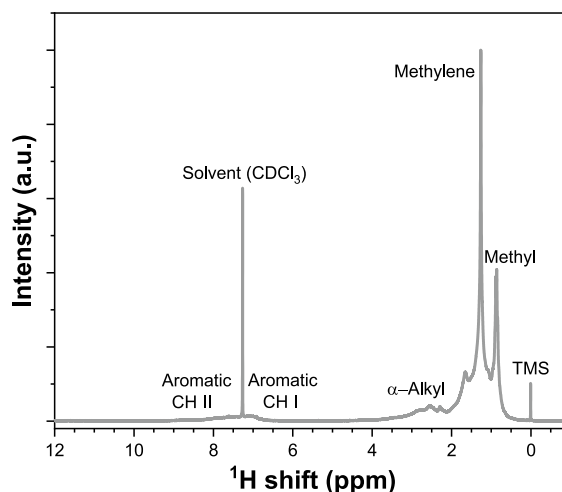
This statistical test is the non-parametric equivalent of the parametric one-way ANOVA test. It is used when the population of two or more independent samples is small (< 15) and shows if the differences between the samples are statistically significant. This is based on the null hypothesis, which assumes that the medians in this case are equal, by being used as a tool of measuring the strength of evidence against the null hypothesis, i.e. the *p*-value with a threshold of significance level set to 0.05 [22]. The threshold of significance value is rather gentle, and modern statistics suggest the *p*-values to be carefully chosen based on the context of the study, the sample size and its effect.

In this work, the Kruskal–Wallis test was employed to show if the changes of the <sup>1</sup>H-NMR results of all 3 labs between their 3 ageing states, using the best practices for data processing for each binder, are statistically significant.

## 4 <sup>1</sup>H-NMR post-processing evaluation

A typical NMR spectrum of bitumen is shown in Fig. 1.

Figure 1 shows that the bitumen spectrum can be separated into four different molecular regions: aromatic CH,  $\alpha$ -alkyl, methylene and methyl groups. When using chloroform (CDCl<sub>3</sub>) as a solvent the quantitative evaluation of the aromatic CH region can be problematic, as the CDCl<sub>3</sub> signal (7.26 ppm) appears in the middle of this region. To minimise the impact of the solvent signal on the quantitative evaluation, the aromatic region was



**Fig. 1** NMR spectrum of bitumen with assigned molecular regions of bitumen, solvent (chloroform, CDCl<sub>3</sub>) and calibration standard (tetramethylsilane, TMS)

split into 2 regions (aromatic CH I and aromatic CH II). The significance of the cut-out area (spectral width = 0.20 ppm, solvent region) was analysed by determining the percentual contribution of this region on the overall aromatic CH region. This was possible by using the data from the laboratory that employed tetrachloroethane (6.00 ppm) as a solvent. The average contribution of this area to the total aromatic region was below 0.20%. Thus, an insignificant amount of information is lost using this approach. Quantification was based on the integrated area of the specific molecular group divided by the sum of all integrated areas (see Eq. 2) and by the number of equivalent hydrogen atoms of the group.

$$\text{Moleculargroup } i(\%) = \frac{\frac{1}{w_i} * A_{\text{Molecular group } i}}{\sum_j^n \left( \frac{1}{w_j} * A_{\text{Molecular group } j} \right)} * 100 \quad (2)$$

Molecular group *i* (%) ...Percentual share of the molecular group *i* (%).

*w<sub>i</sub>*...Normalisation factor for molecular group *i* (-).

*A<sub>Molecular group i</sub>* ...Integration area *A* of molecular group *i* (a.u.)

$\sum_j^n A_{\text{Molecular group } j}$  Sum of the integration areas *A* of all *n* molecular groups (a.u.)

The applied integration borders and the additional normalisation factors were based on literature [13] and are shown in Table 4.

To systematically evaluate the impact of each processing step on the overall error of the small-scale round robin test, the standard deviation ( $\sigma$ ) and coefficient of variation (CoV) were determined iteratively. The laboratories analysed six distinct samples (representing different ageing states), with every sample being measured twice. Each measurement yielded 4 quantitative values corresponding to different molecular groups. To assess reproducibility across laboratories, the values for each molecular group were averaged over all measurements, together with their standard deviation and CV. This procedure was repeated after every processing step, allowing the error progression to be tracked systematically and revealing the influence of each individual step on the overall quantification accuracy.

#### 4.1 Impact of phasing and baseline fitting

In NMR spectroscopy, raw spectral data are obtained as a complex signal, containing both real (a.k.a dispersive) and imaginary (a.k.a absorptive) components. To produce an interpretable spectrum, where signals appear as symmetric peaks on a flat baseline, phase correction is required. This general concept is well established in broader NMR practice [23, 24] but was adapted here to address the particular difficulties presented by bituminous systems, which differ significantly from small-molecule applications. Phasing adjusts the relative contribution of these components, ensuring that peaks are fully in absorption mode and eliminating distortions such as tilted baselines or asymmetric peak shapes.

**Table 4** Integration boundaries for the quantitative evaluation of bitumen NMR spectra

Molecular group i	Lower boundary (ppm)	Upper boundary (ppm)	Normalisation factor (-)
Methyl	0.50	1.00	3
Methylene	1.00	2.00	2
alpha- Alkyl	2.00	4.00	2.5
Aromatic CH I	6.30	7.20	1
Aromatic CH II	7.40	9.00	1

Improper phasing can lead to incorrect peak intensities, shifting chemical shifts, and misinterpretation of spectral features.

Phasing can be particularly challenging in bituminous systems due to the broad and overlapping signals characteristic of these complex mixtures. Unlike well-defined small molecules, where individual peaks are sharp and easily phased, bitumen spectra exhibit broad humps and unresolved regions, making manual phase correction less straightforward. To ensure consistent and reproducible phasing across laboratories, a two-step phasing approach was applied. The signals from solvents, residual solvents (impurities) and reference standards were used as zero-order and first-order phasing references. This procedure resulted in a linear baseline at chemical shifts higher than 10.5 ppm. The 0<sup>th</sup> order phasing was further refined and standardised by extrapolating the linear baseline (between 11 and 13/15/16 ppm) towards the right hill/shoulder of the methyl signal (maximum at 0.88 ppm). This region is very sensitive to 0<sup>th</sup> order phasing (pivot was set to 1.264 ppm, methylene signal) and is raised or lowered significantly by small variations. These adjustments were implemented until the extrapolated baseline intersected at two fixed chemical shifts (10.5 and -0.5 ppm) with the NMR spectrum. These steps extend general phasing concepts by incorporating material-specific features, providing a robust correction method appropriate for bituminous binders. Baseline correction, another established technique in NMR data processing, was also adapted for the specific characteristics of this material class [23]. In general, baseline distortions can arise from instrumental imperfections, external field inhomogeneities, or residual eddy currents, leading to systematic errors in integration. Baseline fitting was then implemented in three different ways:

- 1) Automated baseline fitting based on a polynomial function  $P(x)$  of the form  $P(x) = a_0 + a_1x + a_2x^2 + a_3x^3 + a_4x^4 + a_5x^5$  was implemented.
- 2) Iterative repetition of manual phasing and automatic baseline correction until the baseline was flat and at 0 intensity in the regions  $> 10.5$  ppm, 5.8–4.5 ppm and  $< -0.5$  ppm.
- 3) Baseline fitting based on a linear function extrapolated from the linear region between (11–13/15/16 ppm) through the defined intersection points at 10.5 ppm and  $-0.5$  ppm.



This ensures that the integration of peak areas is not biased by artificial negative contributions, leading to more accurate and reproducible quantitative results. In the context of bitumen, where broad signal envelopes are dominant, such an approach was particularly crucial, as minor distortions can otherwise accumulate and impact spectral interpretation.

Additionally, the dataset was analysed using automatic phase correction and automatic baseline correction. Automatic phase correction refers to an algorithmic approach implemented in TopSpin (Bruker, Karlsruhe) to optimise the phase parameters of an NMR spectrum without manual intervention. The automatic phase correction algorithm iteratively adjusts zero-order and first-order phase parameters by minimising the dispersive signal contribution and maximising the symmetry of absorptive peaks. It evaluates phase shifts across the spectrum and applies an optimisation routine to achieve the best possible correction. This ensures that peaks appear symmetric with a consistent baseline, reducing distortions that could affect spectral interpretation.

#### 4.2 Impact of signal-to-noise ratio

As shown in Table 3, the signal-to-noise (S/N) ratio varied significantly among the participating laboratories. Low spectral resolution and high noise levels can particularly affect the quantification of low-intensity regions. To mitigate these effects, the S/N ratio of bitumen spectra can be enhanced by applying an apodisation function to the free induction decay (FID), a widely used technique in NMR data processing [23]. Apodisation refers to the process of applying a mathematical window function to the FID data to reduce noise and enhance the overall S/N ratio. This function typically involves multiplying the FID signal by a decaying exponential (or another type of window) to selectively attenuate the low-intensity, noisy regions at the tail end of the FID. As a result, noise is reduced, while the high-intensity regions that are less affected by noise retain their original amplitude. This results in an improved S/N ratio accompanied by an increase in peak widths, or line broadening, in the final spectrum.

Although various apodisation functions are available in commonly used NMR processing software (e.g., exponential, Gaussian, Lorentz-to-Gauss, or sine bell functions), they essentially serve the same

purpose of enhancing S/N ratio while trading off spectral resolution. In this study, we chose the exponential approach because it is the most commonly used and should be seen as a representative example to illustrate the impact of this type of data processing. For bitumen analysis, the resulting line broadening from apodisation is negligible, since the intrinsic signals of bitumen are already broad compared to the additional broadening introduced by the mathematical function. Consequently, the slight loss in resolution does not significantly affect the interpretation of bitumen spectra.

To illustrate this, Fig. 2 shows the impact of mathematically induced line broadening on a representative bitumen spectrum, along with its effect on the S/N ratio. The line broadening values (in Hz) applied to the spectra and the corresponding changes in the S/N ratio are summarised in the table within Fig. 2. Equation 3 describes the specific broadening function used in this approach.

$$S'(t) = S(t) * W(t) = S(t) * e^{-\pi * LB * t} \quad (3)$$

$S'(t)$ ...Apodised FID signal (a.u.)

$S(t)$ ...Original FID signal (a.u.)

$W(t)$ ...Window function (a.u.)

LB...Line broadening (Hz).

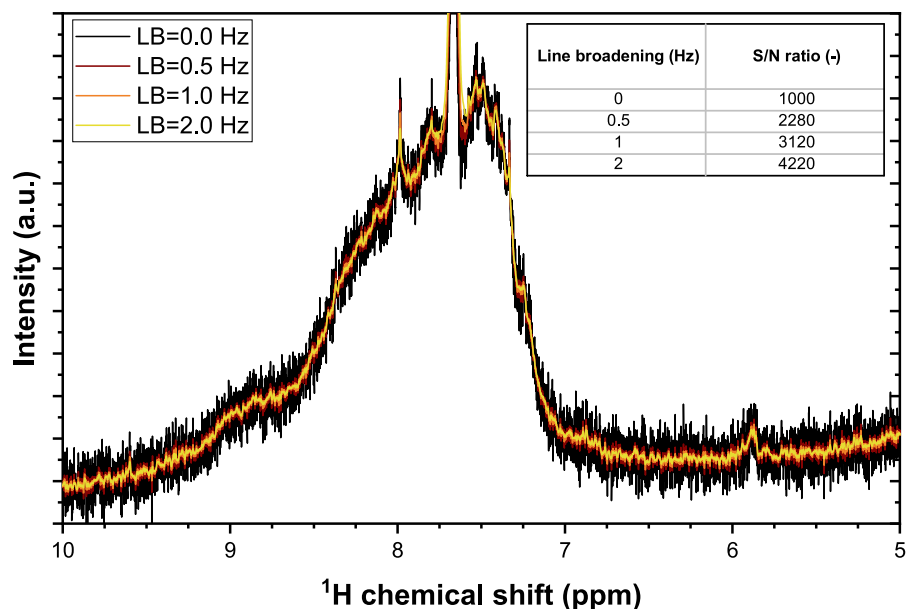
$t$ ...Time-domain variable (s).

#### 4.3 Impact of different calibration approaches

In  $^1\text{H}$ -NMR spectroscopy, chemical shift calibration is crucial for accurate spectral analysis. The most common approach is internal calibration, where a reference compound such as tetramethylsilane (TMS, 0 ppm) or a residual solvent peak (e.g.,  $\text{CDCl}_3$  at 7.26 ppm) is used to set a fixed chemical shift. This method assumes that the spectrometer's nominal frequency is precise, meaning that all chemical shifts scale correctly across the spectrum. However, when analysing datasets from different laboratories, an unexpected systematic deviation was observed. It was observed that after calibrating TMS to 0 ppm, the  $\text{CDCl}_3$  peak did not always appear at exactly 7.26 ppm, with deviations increasing at higher chemical shift values. This observation suggests that small discrepancies between the nominal and actual spectrometer frequency could lead to



**Fig. 2** Impact of additional line broadening (LB) on the signal-to-noise ratio



a position-dependent distortion of chemical shifts. To correct this issue, a refined calibration approach was developed. To this end, the spectrometer frequency was systematically adjusted in small increments, and the distance between TMS and  $\text{CDCl}_3$  was measured for each setting. A calibration curve was constructed, allowing for the determination of the optimal spectrometer frequency where the expected 7.26 ppm separation was achieved. This method ensures that chemical shifts across the entire spectrum are correctly scaled, leading to more accurate integrations and spectral comparisons. This problem is illustrated graphically in Fig. 3. Although the peaks at the beginning of the spectrum are overlapping after the punctual TMS calibration, they spread apart at higher chemical shifts due to the deviations described above. The problem now arises with fixed integration limits, as this shift would attribute a lower quantity to the molecular groups of interest than without this shift. In summary, while internal referencing is common

NMR practice [23], its direct translation to complex materials and cross-laboratory settings requires careful reconsideration.

#### 4.4 Solvents, standards and impurities

Accurate NMR analysis of bitumen requires careful consideration of solvents, internal standards, and potential contaminants. While deuterated solvents are essential for NMR measurements, their residual protonated forms can introduce unwanted signals that may overlap with key spectral regions (e.g. overlapping of  $\text{CDCl}_3$  with aromatic bitumen signals). Additionally, certain impurities, such as water, residual reagents, or degradation products, can appear in the spectrum and interfere with integration or peak assignment. Even internal standards (TMS, 0 ppm) must be used cautiously, as incorrect calibration or solvent interactions can impact spectral accuracy.

**Fig. 3** Potential errors in one-point calibrated NMR spectra of bitumen

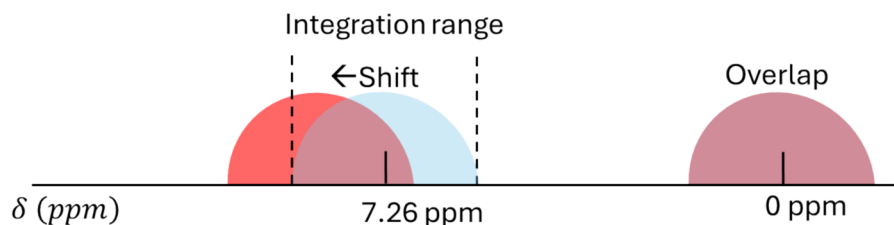


Table 5 provides an overview of common solvents, standards, and contaminants encountered in bitumen research, along with their characteristic chemical shifts. Being aware that these signals are crucial for distinguishing actual sample components from external influences, ensuring reliable data interpretation.

#### 4.5 Impact of delay time

For reliable quantitative  $^1\text{H-NMR}$  spectroscopy, the selection of an appropriate delay time is essential. The delay time represents the interval between successive pulses in an NMR experiment, allowing the nuclei to fully relax back to their equilibrium state before the next excitation. If the delay time is too short, signals from nuclei with longer relaxation times may be underestimated, leading to inaccurate quantification. This effect is particularly relevant in complex organic mixtures, where aliphatic and aromatic structures may exhibit different relaxation behaviours. Aromatic protons generally have longer relaxation times, meaning they require a longer delay time for full signal recovery. If the delay is too short, their signal intensity will be lower than expected, leading to an overestimation of aliphatic components. Unlike many conventional organic systems, bitumen contains paramagnetic species and organic radicals, which can strongly influence relaxation behaviour [26]. These species interact with the nuclear spins, enhancing relaxation and thereby reducing relaxation times. This effect is especially relevant in aged bitumen, where the concentration of radicals increases [27]. The higher radical content further accelerates relaxation, which suggests that the delay time becomes even less critical in aged

samples. Another important factor is the molecular mobility of bituminous molecules/agglomerates. Bitumen does not fully dissolve as individual molecules; instead, it tends to form molecular associations in many solvents (e.g. chloroform, toluene), which are dependent on concentration and solvent polarity [28, 29]. Ageing induces molecular changes such as oxidation, aromatization, condensation, which generally enhance the intermolecular interactions. The impact of these changes can be observed with an increase in content and size of nanoaggregates in the material [30]. Given that all samples are prepared at the same concentration, aged bitumen is expected to form more and larger agglomerates also in solution, leading to reduced proton mobility and thus lower relaxation times. As a result, the overall relaxation in bitumen is significantly faster than in typical organic compounds, which means that shorter delay times may still be sufficient for accurate quantification. [27]. Nonetheless, verifying this experimentally is important to ensure quantitative reliability. To assess the influence of delay time on signal accuracy, an experimental investigation was conducted by laboratory I. Figure 4 presents the results for the bituminous samples B1198A and B1199A, measured with delay times of 1, 10, 20, and 30 s.

The data shows that a delay time of 1 s is sufficient for quantitative analysis in bituminous systems, as negligible changes are observed when increasing the time to 10, 20 and 30 s. This finding is attributed to the high concentration of organic radicals [13], which can significantly accelerate the relaxation processes of excited nuclei in bitumen. Consequently, extending the delay time beyond 1 s does not provide any substantial benefit for quantification. However, if time and facilities allow, relaxation times can be measured accurately using inversion or saturation recovery experiments. These methods provide precise  $T_1$  values, enabling optimal delay time settings, typically 3–5 times the longest  $T_1$ , ensuring accurate quantification even in complex mixtures [23].

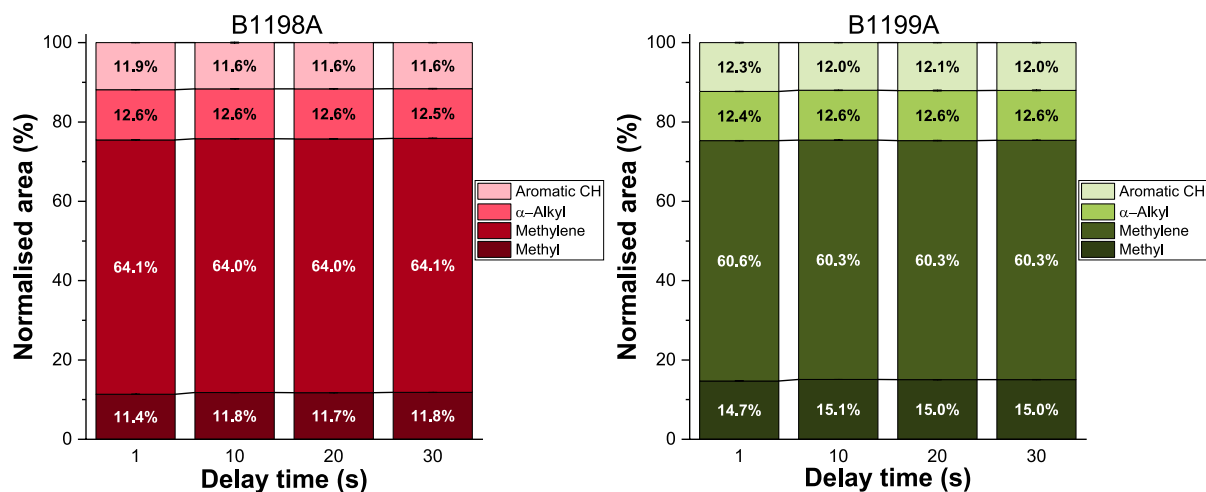
#### 4.6 Error analysis

The error analysis of automatic phasing and automatic baseline correction, manual phasing without baseline correction and combined with three different baseline fitting approaches, as well as the impact of S/N ratio (line broadening) and calibration method,

**Table 5** Identification and chemical shifts of typical solvents, standards and contaminants in bitumen research [25]

Chemical shift (ppm)	Solvent/Contaminant
0.00	Tetramethylsilane (Standard)
0.88, 1.27, 1.30	n-Heptane
1.56	Water
2.17	Acetone
2.36, 7.17, 7.25	Toluene
3.66	Methanol
5.30	Dichloromethane
6.00	Tetrachloroethane
7.26	Chloroform





**Fig. 4** The relative content of aromatic CH, methyl, methylene, and  $\alpha$ -methyl/methylene groups in the binders B1198A and B1199A measured with different delay times (1, 10, 20 and 30 s)

was analysed. The resultant CoV values of the integration areas of the molecular groups for all samples measured by all three laboratories are shown in Table 6.

This study showed that the quantification of bitumen structures with automated phasing and automated baseline correction (see AP + ABC in Table 6) depends strongly on the acquisition parameters, analytes and measurement procedure, leading to very high deviations. Manual phasing considerably improves reproducibility even without any baseline correction. However, the baseline of each laboratory was slightly different after manual phasing. Automatic baseline correction did not improve the reproducibility as the baseline function (polynomial of

5th order) overfits the ‘flat’ spectral areas leading in some cases to spectral regions with intensities less than 0 and significant differences in the most intense signals of the aliphatic region. The procedure of iteratively repeating manual phasing and automated baseline correction (5th order polynomial) to obtain a flat baseline in the regions above 10 ppm, between 5.8 and 4.5 ppm and below  $-0.5$  ppm led to similar results as the combination of manual phasing with singular automated baseline correction. Additionally, a wavy baseline was observed in one laboratory, indicating the potential problem of overfitting in this approach. Therefore, a simpler linear baseline fit based on the extended flat baseline region above 10 ppm (11–13/15/16 ppm) was applied. The fixed

**Table 6** Coefficient of variation (CoV) for every sample after various processing steps. AP=automatic phasing, ABC=automatic baseline correction, MP=manual phasing, MBC=manual baseline correction, LB=Line broadening, Cal=Distance calibration

Sample	Ageing state	AP+ABC	MP (%)	MP+ABC (%)	Iterative MP+ABC (%)	MP+MBC (%)	MP+MBC+LB (%)	MP+MBC+Cal (%)
		CoV (%)	CoV (%)	CoV (%)	CoV (%)	CoV (%)	CoV (%)	CoV (%)
B1198	Unaged	27.49	4.74	3.47	3.21	1.03	1.34	1.00
	STA	28.44	4.79	3.97	3.93	0.88	2.08	1.12
	PAV	28.27	5.57	3.39	3.97	0.75	1.83	0.60
B1199	Unaged	22.83	4.61	2.55	2.17	0.99	1.16	1.52
	STA	27.52	3.64	3.07	2.30	1.09	0.76	1.59
	PAV	26.29	4.79	2.78	2.19	0.80	1.69	1.04
<b>CoV</b>		<b>26.81 ± 2.09</b>	<b>4.69 ± 0.62</b>	<b>3.21 ± 0.51</b>	<b>2.96 ± 0.86</b>	<b>0.92 ± 0.13</b>	<b>1.48 ± 0.49</b>	<b>1.14 ± 0.37</b>



baseline intersections with the graph at 10.5 ppm and  $-0.5$  ppm improved the analysis significantly, indicated by an average CoV smaller than 1% [31].

These processing steps (i.e., manual phasing and manual linear baseline correction) were the starting point to evaluate the impact of S/N ratio and the calibration method. It was possible to improve the S/N ratio by a factor of 4, facilitating a more precise integration and more reliable results that are not distorted by noise. Another important fact that was found while scanning the data was that point calibration could lead to differences in the quantification of bitumen (see Sect. 4.3.). The adjustments made by distance calibration slightly reduced the incorporation of wrong spectral areas, but the overall impact was only minor on the mean values and the CoV of each group.

## 5 Best practices and recommendations

After a systematic evaluation of the sensitivity of data post-processing and the sample preparation factors via this small-scale round-robin, this TG provides some recommendations for the best practices when analysing bituminous binders with  $^1\text{H-NMR}$ .

### 5.1 5.1. Sample preparation

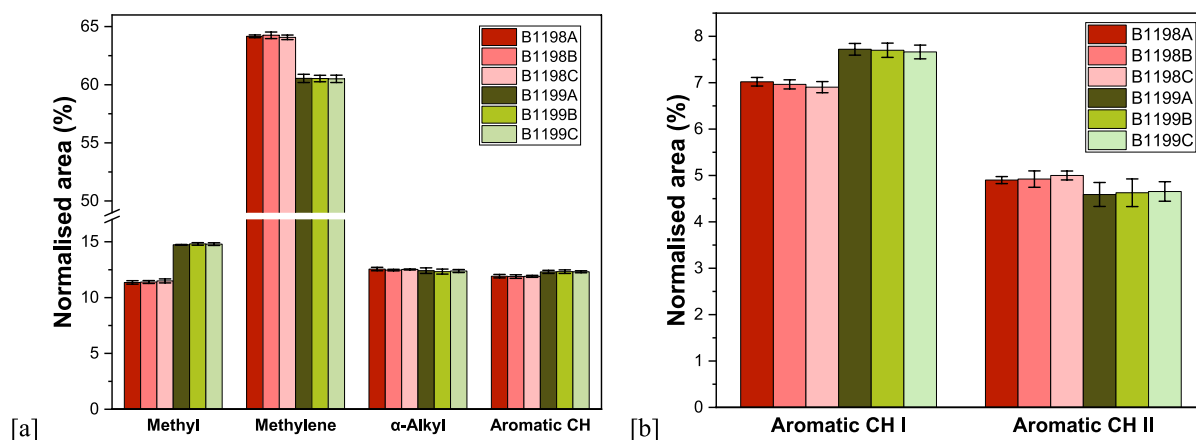
- The use of deuterated solvents, like the ones used in this round-robin, should guarantee complete dissolution of bitumen.
- As chloroform ( $\text{CDCl}_3$ ) overlaps with the aromatic bitumen signals, a way to overcome this obstacle is to separate the aromatic region into two parts, excluding the contributions of  $\text{CDCl}_3$ , as the quantitative analysis of this region in bitumen spectra was found negligible.
- Sample concentration should ensure complete dissolution, which is particularly important for aged bitumen samples, as solubility can vary depending on the degree of ageing. A recommended concentration range is 25–40 mg/mL: concentrations below this lead to poor signal-to-noise ratios (see Table 3), while higher concentrations increase the risk of incomplete dissolution, which can result in biased quantification, as less soluble components may remain undissolved and therefore be under-represented in the NMR spectrum. To verify solu-

bility, preliminary precipitation tests (e.g., spotting the solution on filter paper) using  $\text{CHCl}_3$  are advised prior to NMR measurement.

- Manual phasing of the spectra should consider solvent peaks, reference peaks (TMS), and a flat baseline region above 10.5 ppm. Analysis can be improved by adding low amounts of non-overlapping solvent (e.g. DCM) and TMS to further improve 0<sup>th</sup> order and 1<sup>st</sup> order corrections and by extending the measurement range to 15 ppm and  $-3$  ppm, respectively.

### 5.2 5.2. Data processing

- Characteristic NMR regions should be ideally normalised by the average number of equivalently contributing H atoms of the molecular group (e.g. 3 H atoms in methyl groups). However, as the  $\alpha$ -methyl to  $\alpha$ -methylene ratio and the average number of substituents on aromatic rings are not precisely known, only estimates are possible. Instead  $\alpha$ -Methyl to  $\alpha$ -methylene ratio can be evaluated by employing 2D NMR techniques such as hetero-nuclear quantum coherence spectroscopy. According to literature [32], the average  $\alpha$ -alkyl H atom content is between 2.3 and 2.5.
- No information was found regarding average number of substituents on aromatic rings. Detectable aromatic compounds can contain 1–5 H-atoms (assuming that no benzene molecules are in bitumen). Thus, the choice of 1 H atom per aromatic unit (normalisation factor) probably overestimates the percentual share of aromatic groups within bitumen. Additional measurements and novel approaches (e.g. high-resolution mass spectrometry) are needed to have reliable information on the substitution pattern of aromatic systems.
- Automated phase correction and automated baseline correction are not recommended for bitumen. Instead, it is recommended that phasing is controlled by extrapolating the linear region between ( $\sim$ )15–11 ppm towards the methyl signal and keeping the intersections fixed for all binders at all ageing states. Based on the extrapolation approach, a linear baseline correction is sufficient to obtain accurate and reproducible results.



**Fig. 5** The relative content of methyl, methylene,  $\alpha$ -alkyl and aromatic CH [a] and aromatic CH I and aromatic CH II groups [b] in binders B1198 and B1199 in unaged (A), short-term aged (B) and long-term aged (C) state

- Improving the S/N ratio in postprocessing improves the accuracy of the integration. However, the impact was negligible for the data evaluation of this study.
- The differences in terms of mean values and reproducibility were negligible when changing the calibration procedure. However, care should be taken, as more severe differences between the nominal and actual spectrometer frequency can possibly impact the results. Thus, a simple control of characteristic chemical shifts (e.g. the solvent peak) after point calibration should be sufficient.

### 5.2.1 Case study: molecular fingerprinting and ageing trends

This case study is based on the best practices for sample preparation and data post-processing as recommended by this TG. The analysis of molecular features of the investigated binders and the impact of the standardised laboratory ageing procedures is based on the fully processed spectra (4.2–4.6). The mean values of the percentual share of the molecular groups of each binder were calculated from the NMR spectra of all participating laboratories. From previous experience and comparison with solid-state spectroscopic techniques, the determined errors, if one considers their coefficient of variation as a reliable metric, can be realised as acceptable for a complex organic material, such as bitumen.

NMR spectroscopy helps to clearly distinguish binder B1198 from B1199 due to differences in their methylene/methyl content. The molecular reasons for these observations are variations in the average chain length and/or the branching extent of aliphatic moieties within the system. The smaller the aliphatic chains and the higher the branching is, the more methyls are present (which decreases the methylene/methyl ratio). Thus, the aliphatic chains in binder B1198 are, on average, bigger and less branched than the chains of binder B1199. On the other hand, the aromatic signals (aromatic CH and  $\alpha$ -alkyl) show no significant differences between the binders, suggesting that the aromatic content is similar for both binders. Although the sum aromatic CH region is very similar for both binders, it was observed that the amount of aromatic CH I and aromatic CH II is different and unique for each binder (see Fig. 5b). Possible molecular explanations are differences in the structure of the aromatic systems. According to that, the spectral regions enriched with smaller aromatic systems (aromatic CH I) are higher in binder B1199, while the bigger aromatic systems (aromatic CH II) are slightly more abundant in binder B1198.

The impact of ageing was, in general, statistically insignificant as revealed by the Kruskal–Wallis statistical test in Table 7, with  $p$ -values between the three ageing states much greater than the threshold significance level of 0.05. This means that the null hypothesis is not valid, and the datasets between the three ageing states for all molecular groups are statistically



**Table 7** Non-parametrical analysis of ageing based on the Kruskal–Wallis test

Binder	Ageing	p-value					
		Methyl	Methylene	$\alpha$ -Alkyl	Aromatics	Aromatic CH I	Aromatic CH II
<b>B1198</b>	<b>A, B, C</b>	0.7334	0.3926	0.8106	0.8781	0.5655	0.2645
<b>B1199</b>	<b>A, B, C</b>	0.7430	0.9243	0.5736	0.8557	0.6012	0.5944

similar. Nevertheless, if one focuses solely on the average values, a systematic decrease in smaller aromatic groups (aromatic CH I) and a simultaneous increase in bigger aromatic groups can be seen in both binders, pointing out a reasonable redistribution within the total aromatic group.

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## Declarations

**Competing interest** The authors have no competing interests to declare that are relevant to the content of this article.

## References

- Asphalt Institute Inc. and European Bitumen Association-Eurobitume, The bitumen industry: a global perspective, 2015.
- Pipintakos G, Sreeram A, Mirwald J, Bhasin A (2024) Engineering bitumen for future asphalt pavements: a review of chemistry, structure and rheology. *Mater Des.* <https://doi.org/10.1016/j.matdes.2024.113157>
- Mirwald J, Nura D, Hofko B (2022) Recommendations for handling bitumen prior to FTIR spectroscopy. *Mater Struct* 55:26. <https://doi.org/10.1617/s11527-022-01884-1>
- Hofko B, Alavi MZ, Grothe H, Jones D, Harvey J (2017) Repeatability and sensitivity of FTIR ATR spectral analysis methods for bituminous binders. *Mater Struct* 50:187. <https://doi.org/10.1617/s11527-017-1059-x>
- Hofko B, Porot L, Cannone AF, Poulidakos L, Huber L, Lu X, Grothe H, Mollenhauer K (2018) FTIR spectral analysis of bituminous binders: reproducibility and impact of ageing temperature. *Mater Struct.* <https://doi.org/10.1617/s11527-018-1170-7>
- Giunta CJ, Mainz VV (2020) Discovery of nuclear magnetic resonance: rabi, purcell, and bloch, in: pioneers of magnetic resonance. *American Chem Soc.* <https://doi.org/10.1021/bk-2020-1349.ch001>
- Lu X, Soenen H, Sjövall P, Pipintakos G (2021) Analysis of asphaltenes and maltenes before and after long-term aging of bitumen. *Fuel* 304:121426. <https://doi.org/10.1016/j.fuel.2021.121426>
- Herrington PR, Patrick JE, Ball GFA (1994) Oxidation of roading asphalts. *Ind Eng Chem Res* 33:2801–2809
- Rossi CO, Caputo P, De Luca G, Maiuolo L, Eskandarsefat S, Sangiorgi C (2018) 1H-NMR spectroscopy: a possible approach to advanced bitumen characterization for industrial and paving applications. *Appl Sci.* <https://doi.org/10.3390/app8020229>
- Filippelli L, Gentile L, Rossi CO, Ranieri GA, Antunes FE (2012) Structural change of bitumen in the recycling process by using rheology and NMR. *Ind Eng Chem Res* 51:16346–16353
- Michon L, Martin D, Planche J-P, Hanquet B (1997) Estimation of average structural parameters of bitumens by 13C nuclear magnetic resonance spectroscopy. *Fuel* 76:9–15
- Siddiqui MN (2010) NMR fingerprinting of chemical changes in asphalt fractions on oxidation. *Pet Sci Technol* 28:401–411
- Pipintakos G, Soenen H, Ching HV, Vande Velde C, Van Doorslaer S, Lemiere F, Varveri A (2021) Exploring the oxidative mechanisms of bitumen after laboratory short- and long-term ageing. *Constr Build Mater.* <https://doi.org/10.1016/j.conbuildmat.2021.123182>
- Woods J, Kung J, Kingston D, Kotlyar L, Sparks B, McCracken T (2008) Canadian crudes: a comparative study of SARA fractions from a modified HPLC separation technique. *Oil Gas Sci Technol-Revue de l'IFP* 63:151–163
- Huang J (2010) Characterization of asphalt fractions by NMR spectroscopy. *Pet Sci Technol* 28:618–624



16. Guo M, Liang M, Fu Y, Sreeram A, Bhasin A (2021) Average molecular structure models of unaged asphalt binder fractions. *Mater Struct* 54:173
17. Shikhov I, Thomas DS, Rawal A, Yao Y, Gizatullin B, Hook JM, Stapf S, Arns CH (2019) Application of low-field,  $1\text{H}/13\text{C}$  high-field solution and solid state NMR for characterisation of oil fractions responsible for wettability change in sandstones. *Magn Reson Imaging* 56:77–85
18. Behera B, Ray SS, Singh ID (2008) Structural characterization of FCC feeds from Indian refineries by NMR spectroscopy. *Fuel* 87:2322–2333
19. Elbaz AM, Gani A, Hourani N, Emwas A-H, Sarathy SM, Roberts WL (2015) TG/DTG, FT-ICR mass spectrometry, and NMR spectroscopy study of heavy fuel oil. *Energy Fuels* 29:7825–7835
20. Werkovits S, Bacher M, Theiner J, Rosenau T, Grothe H (2022) Multi-spectroscopic characterization of bitumen and its polarity-based fractions. *Constr Build Mater* 352:128992
21. Werkovits S, Primerano K, Bacher M, Rosenau T, Hofko B, Grothe H (2025) An analytical framework to assess the chemical changes in polymer-modified bitumen upon natural and simulated ageing. *Fuel*. <https://doi.org/10.1016/j.fuel.2024.133257>
22. Jacobs G, Pipintakos G, den Van Buijs X, Kalama DM, den Van bergh W (2023) Chemo-rheological equivalence of bitumen between different lab ageing procedures: from binder to mixture. *Road Mater Pavement Des*. <https://doi.org/10.1080/14680629.2023.2170271>
23. M. Bernstein, B. Diehl, U. Holzgrabe, M. Maiwald, K. Meyer, Y. Monakhova, T. Schönberger, eds, (2024) *qNMR : the handbook*, Books on demand
24. Giraudeau P (2023) Quantitative NMR spectroscopy of complex mixtures. *Chem Commun* 59:6627–6642. <https://doi.org/10.1039/D3CC01455J>
25. Babij NR, McCusker EO, Whiteker GT, Canturk B, Choy N, Creemer LC, De Amicis CV, Hewlett NM, Johnson PL, Knobelsdorf JA, Li F, Lorsbach BA, Nugent BM, Ryan SJ, Smith MR, Yang Q (2016) NMR chemical shifts of trace impurities: industrially preferred solvents used in process and green chemistry. *Org Process Res Dev* 20(20):661–667. <https://doi.org/10.1021/acs.oprd.5b00417>
26. Kocman V, Di Mauro GM, Veglia G, Ramamoorthy A (2019) Use of paramagnetic systems to speed-up NMR data acquisition and for structural and dynamic studies. *Solid State Nucl Magn Reson* 102:36–46
27. Pipintakos G, Ching HV, Soenen H, Sjövall P, Mühlich U, Van Doorslaer S, Varveri A, Lu X (2020) Experimental investigation of the oxidative ageing mechanisms in bitumen. *Constr Build Mater*. <https://doi.org/10.1016/j.conbuilmat.2020.119702>
28. Oh K, Ring TA, Deo MD (2004) Asphaltene aggregation in organic solvents. *J Colloid Interface Sci* 271:212–219
29. Evdokimov IN, Fesan AA (2016) Multi-step formation of asphaltene colloids in dilute solutions. *Colloids Surf A Physicochem Eng Asp* 492:170–180. <https://doi.org/10.1016/j.colsurfa.2015.11.072>
30. Lahjiri F, Mouillet V, Dony A, Ziyani L, Gazeau S, Delfosse F, Sztucki M, Dieudonne-George P, Henn F (2023) Relationships between chemical composition, asphaltene nanostructures, and thermochemical properties of bitumen before and after accelerated oxidative aging. *Energy Fuels* 37:8444–8455. <https://doi.org/10.1021/acs.energyfuels.2c04159>
31. Campbell MJ, Machin D, Walters SJ (2010) *Medical statistics: a textbook for the health sciences*. John Wiley & Sons, Hoboken
32. S. Werkovits (2024) *Elucidation of chemical ageing processes in bituminous construction materials*, Technische Universität Wien

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