Utilising fluorescence spectroscopy and optical microscopy to investigate bitumen long-term ageing

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ABSTRACT

Linking the chemical composition of bitumen to its microstructure is a challenge due to its complexity, viscoelasticity and sensitivity to high energy input. Therefore, fluorescence spectroscopy combined with fluorescence microscopy are promising tools, as they are capable to link the materials fluorescence behaviour to its respective microstructural features. Herein, these techniques were coupled with dark-field microscopy to gain insight on the chemical composition of these auto fluorescent microstructural features and their changes during long-term-ageing (LTA). The results show that upon LTA a loss in fluorescence intensity goes in line with a shift to higher emission wavelength. While the overall intensity decreases during ageing the density and number of fluorescent particles increases. Dark-field microscopy confirms that these fluorescent particles show particle light scattering, proving that on the micro-scale bitumen is a non-homogeneous material.

Introduction

Chemical analysis of bitumen is a challenge due to its natural complexity, temperature dependent viscoelastic properties as well as sensitivity to high energy (high energetic impact, e.g. by electrons or UV radiation, can carbonise and destroy bitumen). As it consists of many different hydrocarbon molecules, a precise chemical composition cannot be given easily (Lesueur, 2009). Standard chemical analysis involving scanning electron microscopy (SEM) coupled with energy dispersive X-ray spectroscopy (EDX), powder x-ray diffraction (XRD), or nuclear magnetic resonance (NMR) (Caputo et al., 2019a; Caputo et al., 2019b; Jennings et al., 1992) are difficult to perform and usually require tweaks to either method or to the sample. Hence, there is a limited number of methods that can be used to investigate bitumen efficiently without biasing the composition or microstructure by sample preparation. The most common method is Attenuated-Total-Reflection Fourier-Transform-Infrared (ATR-FTIR) spectroscopy, where a solid bituminous sample is applied directly onto a crystal interface. This allows for a simple and time efficient analysis of bitumen in different modifications or ageing states (Hofko et al., 2017; Hofko et al., 2018; Lamontagne, 2001). While this method provides information regarding functional groups occurring in bitumen, it can only access the material from a certain perspective. Therefore, other parts of the electromagnetic spectrum can be utilised to investigate the chemical composition of bitumen via UV-Vis spectrophotometry or fluorescence spectroscopy. Soenen et al.
J. MIRWALD ET AL.  

(2016) investigated laboratory aged bitumen dissolved in tetrahydrofuran (THF) with UV-vis absorption and found that absorption at longer wavelengths (~600 nm) increase upon ageing which can be justified by the formation of larger poly-aromatic structures upon oxidation. Hou et al. (2018) and Hung and Fini (2019) have shown similar results, as ageing causes an increase of the absorption bands between 700 and 900 nm, which they attribute to an increase in asphaltene content. Busine et al. (1993) have investigated different bitumen using synchronous excitation emission (SEE) fluorescence spectroscopy. Their findings showed that bitumen exhibits a fluorescence signal in the range between 250 and 600 nm. These intensities can be assigned to different aromatic rings within bitumen, where an increase in wavelength corresponds to an increase in aromatic ring size (1 ring systems: 250–300 nm, 2-ring systems: 300–340 nm, 3 or 4 ring systems: 340–390 nm, > 5 from 390 onwards). The authors claim that aging mainly affects highly condensed polyaromatics. Furthermore, Branthaver et al. (1990) performed size-exclusion chromatography (UV detection at 340 nm) on these bitumen samples. The authors suggest that larger highly aromatic molecules within bitumen, which should in theory possess a high fluorescence intensity, do not show such behaviour due to changes in molecular interactions, which would indicate that quenching effects are occurring during the bitumen ageing process. Handle et al. (2016) investigated the same phenomenon, the fluorescence behaviour of bitumen, by application of excitation, emission and 3D-excitation-emission fluorescence spectroscopy on unmodified, solid bitumen and its SARA (Saturated, Aromatics, Resins and Asphaltenes) fractions, revealing that the aromatic fractions shows the highest overall fluorescence intensity. Grossegger et al. (2018) performed fluorescence spectroscopic measurements as well as SARA separation on an unmodified binder in different ageing states. His findings provide evidence that a decrease in the overall fluorescence signal intensity measured can be linked to a shift in the SARA fractions towards higher polarity. Furthermore, investigations by negative electrospray ionisation Fourier transform ion cyclotron resonance mass spectrometry (ESI(-) FT-ICR-MS) showed a decrease in polycyclic aromatic structures of low polarity, which should be found in the aromatic fraction (Handle et al., 2017). Combining these findings, the assumption can be made that a loss in fluorescence intensity is linked to a decrease in small aromatic structures which are elute within the aromatics, when performing a SARA fractionation, but also due to quenching effects occurring during ageing. Ultimately, this leads to the question why bitumen shows such fluorescence behaviour. Usually poly-aromatic systems are able to fluorescence, as their conjugated $\pi$-electrons are excitable and radiation-less transitions are promoted due to the high number of molecular vibrations. Since bitumen is a material formed from ancient plants and organisms, the occurrence of poly-aromatics is given by its nature. Even though fluorescence spectroscopy has only been used to a limited extent on bitumen as it shows difficulties to standardise due to fluctuations in the spectra, its addition to the chemical analysis kit make it valuable. Furthermore, by combining spectroscopy and microscopy, the spectral information can be linked to the surface morphology. Previous work using a confocal laser scanning microscope (CLSM) (Bearsley et al., 2004; Handle et al., 2016; Lu et al., 2005) or incidental fluorescence microscopy (Mirwald et al., 2019, 2020) have provided insight on the microstructure of bitumen and have found correlation to spectral information. However, no direct correlation of unmodified bitumen fluorescence spectra and its surface was reported. Bitumen usually shows a lower-fluorescent matrix with small highly fluorescent particles embedded. First studies showed that a trend between ageing and mean fluorescence intensity could be observed, while particle number and density can change with ageing state (Mirwald et al., 2020). In addition to fluorescence microscopy, other optical microscopic techniques have been used to characterise bitumen. Ramm et al., (2016) made use of a dark-field microscope to gain a deeper look into the subsurface or bulk of the material. Their study revealed an ant-like structure within bitumen, that show particle light scattering (Mie scattering), indicating distinct particles embedded in a matrix.

This study combines fluorescence spectroscopy and optical fluorescence and dark-field microscopy in the quest to obtain information on the ageing process from a perspective other than FTIR spectroscopy and link it to its respective microstructural features. Therefore, two different laboratory LTA procedures, a standard Pressure Ageing Vessel (PAV) procedure and the newly developed Viennese
Binder Ageing method (VBA) (Mirwald et al., 2020), were compared to a 5 years field aged sample in order to gain insight on how well the laboratory ageing procedures are able to mimic the ageing behaviour from the field. The application of fluorescence and dark-field microscopy should provide answers how the microstructure changes during ageing. Furthermore, it can reveal whether these fluorescent particles are part of a homogeneous material or are actual physical objects (particles), embedded in a matrix.

**Materials and methods**

**Material**

The study investigates a 70/100 penetration graded bitumen at its unaged and three different long-term ageing (LTA) states. The laboratory reference ageing consists of a rolling thin film oven test (RTFOT) followed by a pressure ageing vessel (PAV at 100°C and 2.1 MPa) test, which were performed according to European specifications (CEN, 2007; CEN, 2012).

The second laboratory LTA method was the Viennese Binder Ageing (VBA) method (Mirwald et al., 2020), a newly developed method to simulate bitumen field ageing in the laboratory in a more realistic manner. This is achieved by directly inducing two types of reactive oxygen species (ROS), ozone (O₃) and nitrogen oxides (NOₓ), into the ageing atmosphere, which can be found in the troposphere, near the pavement surface (Atkinson, 2000). Their formation in the ageing setup is achieved by leading compressed air through an ozone generator (Anseros ozone generator COM-AD-1) at 1 L/min. A 0.5 mm thin bitumen film is placed in the ageing chamber (Vol.: 10 L), where it is exposed to the ROS enriched atmosphere for nine days at +80°C. A high ROS concentration (O₃: 12 g m⁻³ and NOₓ: ~1.2 g m⁻³) was selected for the ageing procedure to maximise the oxidation potential of the ROS, inducing as much ageing as possible at said temperature and duration. The choice of temperature can be justified, as no thermal ageing occurs at +80°C (Mirwald et al., 2020), also maximising the ageing rate as much as possible, without inducing any thermal ageing or altering the reactions that could occur at too high temperatures (Petersen, 2009). Furthermore, it needs to be kept in mind that the current VBA setup simulates nighttime ageing, as no UV or visible light is present during ageing. The optimisation by implementation of UV light will follow in the future. Prior to VBA, sample preparation as well as short-term-ageing is required. Merging these tasks, 7.7 g of unaged binder is heated up to 150°C for a maximum of 3 min and poured into a metal PAV container with a diameter of 14 cm. Afterwards, the sample holder is placed in a ventilated oven for 75 min at 163°C to guarantee the formation of an even, short-term aged bitumen film. After nine days of ageing at the parameters mentioned above, the sample container is again placed in an oven at 163°C for a maximum of 10 min to melt, homogenise and collect the binder.

The third LTA bitumen investigated was a 5 years field-aged binder that was recovered and extracted from an asphalt mix test field constructed in 2012 (Hofko et al., 2014) according to the EN 12976-3 (CEN, 2013) using tetrachloroethylene. As the test field showed an extensive ageing gradient, merely the binder recovered from the top 1 cm of the hot mix asphalt (HMA) slabs after 5 years of exposure to the atmosphere was selected as the field ageing reference. It is important to note that even though all these LTA bitumen samples represent a different ageing states, their preparation and handling was set to the same conditions (thermal history during preparation). Hence, the microstructures observed are in regards to a homogenised material in the respective ageing state. The parameters applied during sample preparation can be found below.

**Methods**

Since spectroscopy and microscopy are merely analysing the surface of the material, the sample handling and preparation procedure needs to be conducted with special care, compared to mechanical analysis, where not only a larger fraction of molecules is investigated but also the bulk properties are
Table 1. Fluorescence spectroscopy parameters for excitation scans and 3D maps.

| Parameter                  | Excitation scan | 3D Map      |
|----------------------------|-----------------|-------------|
| Excitation Wavelength      | 230–500 nm      | 230–500 nm  |
| Emission Wavelength        | 520 nm          | 400–700 nm  |
| Scan Step Size             | 1 nm            | 5 nm        |
| Repeats per measurement    | 3               | 1           |
| Repeats per Cycle          | 5               | 1           |
| Dwell Time                 | 0.5 s           | 0.5 s       |
| Temp                       | 23–25°C         | 23–25°C     |
| Iris                       | 100%            | 100%        |
| Filter                     | 495 nm          | 420 nm      |
| Scan Slit                  | 2 nm            | 2 nm        |
| Fixed/Offset Slit          | 2 nm            | 2 nm        |

For each ageing state three samples were prepared and measured with five repeats per cycle and three repeats per measurement. To simplify these five spectra per sample, a mean curve was generated, resulting in three mean curves per sample as well as a total mean curve of all three samples per ageing state. These three mean curves per sample and the overall mean curve of the ageing state were plotted using OriginPro2016R and are shown in the results (Figures 1–4).

The fluorescence and dark-field microscope consist of a Nikon Eclipse Ci-L in incident light geometry. It is equipped with a 100 W metal-halide light source (fluorescence light source) and a 50 W halogen lamp (dark-field light source), a colour-digital camera (DS-Fi3), a Plan Fluor 100× objective as well as a universal epi illuminator 2 for dark-field and fluorescence with an excitation filter at 465–495 nm, a dichroic mirror at 505 nm and an emission filter at 515–555 nm (FITC Filter Block). All images were recorded with the respective microscopic software NIS Elements BR. The captured images were used after post-processing by increasing contrast and brightness to provide better visibility. The statistical evaluation of the fluorescent particles was conducted using ImageJR Fiji. For such particle analysis, a background colour threshold adjustment was made. Here, the background (lower fluorescent matrix) of interest. Hence, during sample preparation parameters like heating temperature and time are of critical importance and were applied equally for each sample.

For sample preparation, 3–5 g of bitumen in the respective ageing state was heated up between 150°C and 180°C for a maximum of 2–3 min. This enabled sufficient homogenisation of each sample, which is a key factor for sufficient repeatability and reproducibility of chemical and morphological analysis. During heating, temperature control was achieved by stirring with a thermometer. Once the bitumen has reached a liquid like state, it was poured into three silicone moulds (25 mm DSR form). The three samples within the silicon mould were covered with microscopic slides and left to cool down to room temperature for 20 min, before the silicone moulds were removed, revealing even sample surfaces. The samples were then stored between 1 and 3 h, before the spectra were recorded. This preparation technique and resting time were chosen in regards to a previous study, where the most stable signal was generated when being measured 1–3 h after preparation (Mirwald et al., 2019). As fluorescence microscopy at high magnification (100×) causes high light intensity on a spot, ‘bleaching out’ effects can occur on the sample surface. To minimise the impact, spectroscopic measurements were conducted before microscopy.

An Edinburgh Instruments FPS920 photoluminescence spectrometer was used for recording the fluorescence spectra. The setup consists of a XE900 Xenon Arc Lamp (500 W) as an illumination source, double Czerny-Turner monochromators (type TMS300) at both excitation and emission arms and a S900 single-photon photomultiplier (type R928) detector. Two different types of spectra were recorded: Excitation scans and 3D emission maps. While the excitation spectra can run more repeats at one specific emission wavelength resulting in a higher quality spectrum, the 3D maps provide a broad overview of a certain emission and excitation wavelength range. Their respective parameters are shown in Table 1.
was set to appear in black and the highly fluorescent particles in white. This allows subsequent particle analysis, revealing their particle number, size and density. For each ageing state, images of all three samples from spectroscopy were recorded and analysed. As these results depend on the threshold set for each image, the data and trends should be taken with care. Nonetheless, they indicate significant changes occurring due to LTA, which are clearly visible in the fluorescence and dark-field images provided in the results at Figures 8 and 9.
Figure 4. Excitation spectra of the 5 years field aged 70/100 binder in its absolute (left) and normalised intensity (right).

Results and discussion

Fluorescence spectroscopy

Figure 1 depicts the excitation spectra of the unaged 70/100 bitumen. The left side shows the mean spectra of all three samples (three repeats per measurement and five repeats per cycle) and the overall mean curve in absolute intensity. This provides information on the overall fluorescence behaviour and intensity of the material at the given parameters. Good repeatability and reproducibility are given for all three samples, as the normalised spectra on the right side show little differences. The unaged bitumen spectra exhibit two distinct maxima at 265 and 300 nm as well as three shoulders at 330, 450 and 480 nm. The highest fluorescence intensity is found at 265 nm, reaching a maximum intensity of 80,000 counts per second (cps). Considering the assignment of different aromatic ring sizes from Buisine et al. (1993), the maximum at 265 and 300 nm could be assigned to a 1 aromatic ring system, whereas the shoulder at 330 nm could represent a 2 ring aromatic system. The shoulders at 450 and 480 nm would resemble > 5 aromatic ring system. It needs to be questioned and investigated if the results from these synchronous excitation emission scans can be compared to the herein performed excitation scans, as they were recorded at a fixed emission wavelength of 520 nm. Nonetheless, both studies outline the fact that the fluorescence behaviour (emission and excitation) of bitumen hanges in regards to its composition (size of aromatic rings and molecular structure). However, a precise molecular structure (precise aromatic ring size and possible sidechains) of these maxima and shoulders cannot be given yet, as merely the absolute changes due to ageing were in scope of this paper. Further possibilities to investigate and simplify an assignment will be discussed at the end of this chapter, when comparing the different spectra (Figure 5).

Looking at the excitation spectra from the RTFOT + PAV aged bitumen (Figure 2), differences in the absolute intensity can be observed across the three samples. This can be explained by the difficulties in providing completely homogeneous sample surfaces, even after using the sample preparation technique with the silicon moulds. However, the maxima and shoulders within the spectrum remain at the previously mentioned positions. Merely their intensity ratios are changed compared to the unaged spectra (as comparison in Figure 5 will show). Furthermore, the maximum fluorescence intensity decays to 35,000 cps (from 80,000 cps). After normalisation, small difference in the mean curves can be seen, indicating that even though the absolute intensity of the three samples differs, their normalised spectra provide sufficient reproducibility. This outlines the necessity to perform normalisation after recording excitation spectra on various samples with the same ageing state.

The excitation spectra of the VBA aged bitumen are displayed in Figure 3. Two of the three samples exhibit a higher absolute fluorescence intensity. However, the sample with the lower intensity (sample 3) shows no differences after normalisation. The overall maximum fluorescence intensity decays...
to 25,000 cps, which is significantly below the RTFOT + PAV aged bitumen (35,000 cps). Hence, the VBA aged bitumen has lost more small aromatics during its ageing process, which are responsible for the fluorescence signals. The previously mentioned maxima and shoulders are again observable. However, the second maximum at 300 nm has transformed into a shoulder, as the entire spectrum exhibits a lower intensity between 265 and 500 nm, compared to the spectra of the RTFOT + PAV aged bitumen.

Figure 4 shows the excitation spectra of the 5-year field aged bitumen. Its overall maximum intensity (22,000 cps) is the lowest of all three LTA bitumen, indicating the overall highest loss in the absolute fluorescence intensity and hence highest ageing level. Small difference in the absolute intensity can again be neglected after normalisation. All maxima and shoulders appear at the previously described wavelengths.

A comparison of the absolute and normalised excitation spectra of the unaged and three LTA bitumen is plotted in Figure 5. This overview shows how the different LTA methods affect the absolute fluorescence intensity level and the respective maxima and shoulders.

Here, the previously mentioned trends of the absolute intensity are well observable, which indicate that the RTFOT + PAV aged binder (35,000 cps) is less aged compared to the VBA (25,000 cps) and field aged binder (22,000 cps). Considering the assignment of wavelength to aromatic rings from Buisine et al. (1993), no clear statement can be given whether single, double or even more complex aromatic rings are decreasing particularly during LTA, as the entire region between 230 and 500 nm is dropping in its absolute intensity. However, looking at the normalised spectra, the field aged binder shows the highest intensity at the local maximum around 300 nm, followed by the RTFOT + PAV and VBA aged sample. Here, the question can be raised if these discrepancies in the spectra are actually coupled to differences in the ageing mechanism, leading to a different content in aromatic ring structures of larger sizes. This would mean that the laboratory aged binders degrade aromatic rings with a larger size (2, 3, 4 and 5 ring systems), compared to the field aged sample. However, this statement needs to be treated with care, as the assumption is based on the normalised spectra at the given parameters and it is not entirely clear whether the selected excitation parameters reflect the materials ageing performance (as the 3D emission maps in Figure 6 will reveal). Furthermore, it needs to be kept in mind that quenching effects of bitumen are not considered as it is not understood yet how they impact the change in the fluorescence signal. However, the differences in the ageing conditions can and need be outlined. While the field aged sample experienced normal atmospheric ageing conditions, VBA, in its current form, simulates night time conditions (Mirwald et al., 2020), as no UV or sunlight was involved and the PAV merely induces thermal ageing under high pressure. As reported in literature (Mary et al., 2013), UV radiation can cleave molecular bonds like the C = C double bond, which can impact the aromatic content and ultimately the intensity of the bands appearing in the spectra.
Moreover, the resting time after preparation can have an impact on the results of the spectra as the sample surfaces need time to settle, which again is linked to their viscosity. While microscopic samples are usually stored for 24 h to allow microstructures to establish (Nahar et al., 2013), spectroscopic measurements are usually performed within hours after preparation (Mirwald et al., 2019). As this study intends to link the microscopic information to the respective spectroscopic results, images were captured directly after recording the spectra. Future studies will look into the spectroscopic and microscopic correlation of resting time and formation of microstructures on the surface of bitumen and whether chemical information changes with formation of such microstructure.

All in all, these excitation spectra suggest that the fluorescence behaviour during ageing cannot be so easily assigned to certain aromatic ring structures, as changes in molecular interaction and quenching need to be considered as well. However, there are ways to provide a broader overview of the fluorescence behaviour of the material: 3D emission–excitation maps, which are depicted in Figure 6. These maps show again the same ageing trends and loss in absolute intensity, ranking the three LTA binders as follows: RTFOT + PAV \((1.35 \times 10^4)\) \(<\) VBA \((1.02 \times 10^4)\) \(<\) Field aged \((0.99 \times 10^4)\). Beside the overall decrease in fluorescence intensity, a shift to higher emission wavelength and lower excitation wavelength (green and turquoise area) occurs between unaged and all LTA states. The extent of the shift again depends on the ageing level.

Comparing the three different LTA ageing procedures, a shift of the maximum (red/orange area) can be linked to an increasing ageing level, as shown in Figure 7. This could provide a link to results from UV-vis spectrophotometry (Hou et al., 2018; Hung & Fini, 2019; Soenen et al., 2016), where an increase in larger aromatic structures results in a shift of the absorption to higher wavelengths. While this again helps to classify the ageing level of the three LTA binder, it shows that the emission wavelength at 520 nm from the excitation scans were not ideal, as this shift due to aging does not capture.

**Figure 6.** 3D map of the unaged and three LTA 70/100 bitumen.
Figure 7. Shift in the 3D maps of the long-term-aged binder.

the changes. Therefore, future investigations on the matter should look into higher emission wavelengths or even application of emission scans. In addition, a follow-up study of the SARA fractions of these specific bitumen samples could provide more information on this matter. Such a separation will reduce the number of molecules present within each fraction which could enable sharper signals and a more specific assignment of the respective fluorescent molecules. Furthermore, it could provide more information on how these LTA binders differentiate on the molecular level and show if the reactive oxygen species from VBA induce a different kind of ageing compared to the thermal/pressure conditions in RTFOT + PAV and ultimately show how it compares field ageing.

Fluorescence and dark-field microscopy

Figure 8 depicts the fluorescence microscopic images of all four investigated bitumen samples. These images provide a link between the fluorescence spectra and the respective sample surface and should help to understand the ageing process and its impact on microstructure and chemical composition. This demonstrates the major advantage of an optical incident fluorescence microscope, as it provides morphological and chemical information of the actual surface at the same time. The addition of dark-field microscopy provides further confirmation on these microstructures as it allows the detection of light scattering particles. This intends to answer the questions whether these fluorescing particles are actual light scattering particles with a high auto fluorescence that are embedded in the lower fluorescent matrix (2-phase system) or whether they are part of a homogeneous (1-phase) system with differences in fluorescence behaviour.

Looking at Figure 8, it is important to note that the brightness and contrast of these images has been adjusted in order to provide good visibility of the microstructural features. The overall fluorescence intensity from microscopy (not presented in this paper) show the same trends as seen in spectroscopy (as reported in a previous study by Mirwald et al. (2020)). Comparing the three LTA binders, the assumption is that with higher ageing level an increase in particle number can be recorded. Looking at the results after integration (see Table 2), VBA shows the higher particle number, average size and particle density (%Area), followed by the field aged and PAV aged binder. As these numbers are
Figure 8. Fluorescence microscopic images of the unaged and three LTA 70/100 bitumen.

Table 2. Results from integration of the fluorescence microscopic images.

| Sample       | Particle Number | Average Size | Density (% Area) |
|--------------|-----------------|--------------|------------------|
| Unaged       | 785             | –a           | 2.9              |
| RTFOT + PAV  | 1006            | 18.1         | 5.8              |
| VBA          | 1240            | 19.9         | 7.8              |
| Field Aged   | 1210            | 17.4         | 6.7              |

*aThe average size of the particles in the unaged samples were not conclusive, as an inhomogeneous illumination profile falsified the values. Hence, they were left out.

dependent on the imaging processing and difficulties in background adjustments, the results of such an integration should be treated with care. However, they give an outline to the overall trend, which shows good accordance when comparing them to Figure 8.

In order to prove that the fluorescing particles are not part of a homogeneous (1-phase) system but actual physical, light scattering particles with boundaries embedded in a matrix, dark-field microscopy was performed on all investigated bitumen samples (see Figure 9). Since dark-field microscopy only detects scattering light near the sample surface, a homogeneous material would not provide any meaningful results. However, since bitumen exhibits distinct microstructures, such a scattering is observable. These scattering centres show identical overlap with the fluorescing particles from Figure 8, confirming that these fluorescing particles poses different optical properties than the matrixes. This provides literal ‘solid’ evidence, that bitumen exhibits distinct particles that are responsible for the fluorescence signal. It is left unanswered how these particles change chemically during ageing or how the overall decrease in fluorescence intensity (shift in the polarity gradient) affects the microstructure beside the observed trends. One approach to tackle such question could be to blend different SARA fractions and see how they contribute to the formation of such particles (in addition to
the single phase analysis). Furthermore, a proper correlation between shift in the emission wavelength (by selection of different excitation scan parameters) and increase in fluorescence particle number and density will be looked into.

In addition to these findings, another critical question can be raised on how these fluorescent microstructures can be linked to the commonly reported bee structures of bitumen (Loeber et al., 1996; Masson et al., 2006). However, as these bee structures are usually reported after a certain resting period (Nahar et al., 2013), further studies on the formation of such bee structures are required, which needs to be coupled with optical dark field and fluorescence microscopy. Therefore, such questions will be tackled in future work, as this study focused solely on the connection to its respective spectroscopic measurements, which was performed 1–3 h after preparation, where the most stable signal is generated.

**Conclusion**

Fluorescence spectroscopy and optical microscopy (dark-field and fluorescence) have been combined to investigate the ageing behaviour of an unaged and the three following LTA 70/100 bitumen:

- RTFOT + PAV aged binder according to European standards (CEN, 2007; CEN, 2012)
- A VBA aged binder which intends to provide a more realistic ageing simulation be implementation of factors from the atmosphere (Ageing parameters: 80°C, 9 days, ROS concentration: 12 g m\(^{-3}\) O\(_3\) and 1.2 g m\(^{-3}\) NO\(_x\), 1 L/min flow rate, 0.5 mm binder thickness) (Mirwald et al., 2020)
- A 5-years field aged binder recovered from the top 1 cm of HMA slabs test field constructed in 2012 (Hofko et al., 2014)
The spectroscopic and microscopic results link chemical composition (fluorescing molecules) to microstructural features within bitumen. By investigation of various LTA samples, the following conclusions can be drawn:

- The absolute fluorescence intensity of the LTA binders indicates that the RTFOT + PAV aged sample is significantly less aged than the VBA and field aged samples.
- The VBA and field aged sample exhibit a similar absolute intensity at the maximum, but are showing differences in the range between 300 and 500 nm which are explained by possible differences in the ageing conditions (night time in VBA versus field conditions) or by non-ideal choice of measurement parameter.
- A specific assignment of observed bands to a molecular structure is difficult, as various effect, like quenching, are not understood yet. However, classification into regions with aromatic rings of different sizes were interpreted and discussed.
- This provides first suggestions that the two laboratory LTA methods lead to a higher degradation of larger aromatic structures, compared to field aged bitumen.
- Fluorescence microscopy shows small fluorescent particles embedded into a lower fluorescent matrix, which increase in number and density upon ageing.
- This increase can be observed for all three LTA binders, with VBA showing slightly higher values than the field and RTFOT + PAV aged sample.
- Dark-field microscopy links the chemical information on these highly fluorescent particles to their physical properties (scattering), confirming that they are distinct particles embedded in a lower fluorescing bitumen matrix.

The combination of both microscopic techniques and spectroscopy provides new insight on the aromatic molecules and link it to morphological features on the surface and subsurface of bitumen. While microscopy investigates samples after a certain resting time, spectroscopy conducts measurement within 1–3 h after preparation. However, since microscopy was performed right after spectroscopy, further studies are required to investigate on this resting time which leads to the formation of such microstructures and how this affects chemical information form the surface. Furthermore, proper correlation to link these shifts due to ageing to changes in the microstructure provide ideas for future studies on such matter. Overall, this should help us to understand the phenomenon of microstructural formation within bitumen and its connection to chemical composition.

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