Direct observation of the asphaltene structure in paving-grade bitumen using confocal laser-scanning microscopy

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Summary
The structure of the asphaltene phase in the bitumen is believed to have a significant effect on its rheological properties. It has traditionally been difficult to observe the asphaltene phase in unaltered samples of bitumen. The maltenes are thought to form a continuous phase in which the asphaltenes are ‘dispersed’. In this study, confocal laser-scanning microscopy (CLSM) operating in fluorescence mode was used to examine the structure of paving-grade Safaniya and San Joaquin bitumen. The asphaltene fraction fluoresces in the 515 – 545 nm wavelength range when irradiated with light with a wavelength of 488 nm. The major advantages of CLSM are that the bitumen sample requires little pretreatment or preparation that may affect the original dispersion of asphaltenes and the bitumen is observed at ambient temperature and pressure. This reduces the possibility of producing images that are not representative of the original material. CLSM was able to show the distribution of maltene and asphaltene components in bitumen. The asphaltene aggregates in the bitumen were observed to be 2–7 µm in size and formed a dispersed ‘sol’ structure in the continuous maltene matrix rather than a network ‘gel’ structure. Surprisingly, the structure and fluorescence of the asphaltene phase does not appear to alter radically upon oxidative ageing. The structure of the asphaltene phase of an AR4000 San Joaquin bitumen was found to be more homogeneous than that of Safaniya bitumen, illustrating the range of structures that can be observed in bitumens by this method.

Introduction
Bitumen is the residue from the vacuum distillation of petroleum oil. The bitumen’s partial solubility in a non-polar solvent can be used to separate it into two broad fractions: asphaltenes and maltenes. The asphaltenes are defined as the bitumen fraction that is insoluble in n-heptane. Asphaltenes are polar with a higher molecular weight than maltenes and are composed of conjugated polyaromatic and polynuclear ring systems. The maltenes are thought to form a continuous phase in which the asphaltenes are ‘dispersed’. The asphaltene fraction has a tendency to aggregate into micelles and form networks and it has long been recognized that it is not only the amount, but also the compatibility between the asphaltene and maltene phases that has a major effect on the bitumen’s rheological properties (Heithaus, 1960; Petersen, 1984; Morgan & Mulder, 1995). The extent to which the highly aromatic, polar asphaltene phase associates with itself has a significant impact upon the physical performance of the bitumen. ‘Gel’ bitumens are highly associated and contain a greater proportion of asphaltenes. They exhibit different rheological properties from ‘sol’ bitumens, which usually contain fewer, less associated asphaltenes.

Lieber et al. (1998) point out that ‘it seems important to leave the colloidal structure of the bitumen system intact when structural observation investigations are undertaken.’ A number of factors can affect the structure of the asphaltene phase, including the temperature and the chemical environment (Heithaus, 1960; Peterson, 1984; Lesueur et al., 1997). A change in the temperature or chemical composition can force associations (or disassociations) amongst the asphaltene molecules that are not present in the original bitumen sample.

Optical, electron and scanning probe microscopy techniques have all been used previously to observe the structure of the asphaltene phase. Simple reflected or transmitted light microscopy has not normally been successful due to the black or dark brown colour of bitumen, which prevents any contrast...
between the asphaltene and maltene phase from being observed. However, Li & Wan (1995) have managed to use optical microscopy to observe the particle size of asphaltenes that were precipitated from bitumen by using a low-molecular weight solvent.

Scanning electron microscopy (SEM) and environmental scanning electron microscopy (ESEM) have both been used successfully to image the structure of the asphaltene phase (Loeber et al., 1996, 1998; Rozeveld et al., 1997; Michon et al., 1998). Although the resolution of electron microscopes is excellent, they do have some shortcomings. For example, SEM requires the use of a vacuum and depress temperature, which can cause a change in the asphaltene structure compared to that at ambient temperature and pressure. SEM and ESEM are also designed to image surface topography, whereas the rheological properties of the bitumen are determined by the internal structure in the bulk of the sample.

Electron microscopy techniques also require extensive sample preparation, which may introduce artefacts into the imaging process. For example, Loeber et al. (1996, 1998) used a solvent leaching technique and Michon et al. (1998) and Rozeveld et al. (1997) used a beam vaporization technique to remove the oily maltene phase from the sample so that the asphaltene structure could be observed. While excellent SEM and ESEM images have been obtained, it is not known what effect the solvent leaching and electron beam vaporization processes have on the asphaltene structure. It is possible that because the asphaltenes were effectively isolated from the maltene fraction and were consequently in a different chemical environment, associations (or disassociations) may have occurred that were not present in the original sample.

Although transmission electron microscopy (TEM) has been used to examine the dispersion of polymer in bitumen (Collins et al., 1991), no literature was found on the use of TEM for examining the structure of the asphaltene phase.

Atomic force microscopy (AFM) is gaining in popularity for examining bitumen samples. The advantage of AFM is that it requires no sample preparation and operates under ambient conditions. Loeber et al. (1996, 1998, 1999) and Pauli et al. (2001) used AFM to observe structures on the surface of a film of bitumen, which they attributed to asphaltene networks. Unfortunately, when used in the imaging mode, AFM reveals only surface features and these may not be representative of the internal structure of the bitumen.

One technique that shows excellent promise for examining the asphaltene network structure in situ is fluorescence microscopy using a confocal laser-scanning microscope (CLSM). Under ideal conditions, CLSM allows structures as small as ~200 nm to be observed (Rowland & Nickless, 2000). The bitumen is observed at ambient temperature and pressure and needs none of the preparation required by electron microscopy. Rather than just observing the surface in a similar manner to SEM, ESEM, AFM and conventional reflected light microscopes, CLSM takes an optical cross-section of the bulk of the sample to be imaged. Furthermore, CLSM does not image objects that are above or below the focal plane so that only those objects that are in focus are recorded. CLSM can operate in the conventional reflected light mode or as fluorescent microscopes.

The current literature on the fluorescence properties of bitumen is contradictory. It has been claimed that bitumen does not exhibit much fluorescence and that under fluorescence microscopy the bitumen appears dark (Collins et al., 1991; Loeber et al., 1996; Newman, 1998). Collins et al. (1991) noted that under blue and UV light with a wavelength of 400–440 nm, the aromatic and resin constituents of the bitumen fluoresce, whereas the asphaltenes and saturates do not. The fluorescent light was filtered so that only wavelengths greater than 470 nm were observed. Loeber et al. (1996) stated that ‘oil exhibits autofluorescence when irradiated with shorter wavelength light such as UV light, but for asphalt there is little fluorescent light emission because the oil phase is mixed with an asphaltene and a resin phase which do not exhibit any autofluorescence.’ However, Loeber et al. (1996) used fluorescence microscopy to observe the presence of dark ‘round forms’ with a diameter of 2–50 µm, which were attributed to asphaltene aggregates dispersed in a slightly fluorescing maltene matrix. Newman (1998) observed that bitumen appears dark grey or black under UV light at 50 × magnification. It may be worth noting that neither Loeber et al. (1996) or Newman (1998) mentioned the use of filters for screening out reflected light when undertaking fluorescent microscopy. It is possible that the reflected image obscured any fluorescence that occurred in the bitumen.

Contrary to the results published by Collins et al. (1991), Loeber et al. (1996) and Newman (1998), the fluorescence properties of asphaltenes were used by Li & Wan (1995) to observe their particle size against a dark bituminos background. The incident light source had a wavelength of 568 nm. They mentioned that ‘since highly conjugated aromatic structures are a major part of asphaltene components, fluorescent excitation by visible light is a distinct possibility.’

Guibault (1990) noted that aromatic hydrocarbons, especially polyaromatic hydrocarbons (PAH), generally exhibit fluorescence in the UV or visible spectrum. The wavelength at which maximum absorbance and fluorescence occurs tends to increase as the level of conjugation increases.

Mikula & Munoz (2000) used CLSM to observe that bitumen and oil exhibit fluorescence when irradiated with 488 nm wavelength light. Furthermore, fluorescence has been used extensively in the petrological examination of bituminous materials such as coal, shale oils, natural petroleum and asphalt (Rost, 1995).

The purpose of this investigation was to examine the asphaltene structure of paving-grade bitumen in situ, using a CLSM operating in fluorescent mode. Although fluorescence microscopy has been used extensively to examine the dispersion of polymers in bitumen and bituminous emulsions (Collins
et al., 1991; Lee et al., 1997; Rozeveld et al., 1997; Newman, 1998; Takamura & Heckmann, 1999; Forbes et al., 2001), little information exists on the use of CLSM and fluorescence microscopy for examining the asphaltene structure in unmodified paving-grade bitumens. The major advantage of CLSM in this application is that the bitumen sample requires little pretreatment or preparation that may affect the original dispersion of asphaltenes in the bitumen.

Materials and Methods

Paving-grade bitumens are commonly classified by their penetration value at 25 °C or their viscosity at 60 °C. The penetration value is the distance (measured in tenths of a millimetre) that a 100 g needle of defined dimensions penetrates into a bitumen sample at 25 °C, when it is allowed to fall under the influence of gravity for a period of 5 s. Hence a 180/200 grade is softer than an 80/100 grade. Three paving-grade bitumens were used during this study: two penetration-graded bitumens and a viscosity-graded bitumen. The two penetration-graded bitumens were obtained from a Saudi Arabian, Safaniya crude. The 180/200 penetration-grade bitumen was a straight-run vacuum residue, whereas the 80/100 grade bitumen was partially air-blown. The bitumens were fractionated into their asphaltene, resin (polar aromatic), aromatic (naphthene aromatic) and saturate fractions using the method described by ASTM D4124-01 (2002). The asphaltene fraction was recovered by filtration using a Whatman GF/A filter paper. The resin, aromatic and saturate fractions were recovered by dissolving them in toluene and placing the solution into a BP-FTIC filter was used to observe the fluorescence in the 515–545 nm wavelength range. All images were captured as two-dimensional images in 512 × 512 TIFF format.

Results

Fluorescing regions can be seen clearly in each of the CLSM images of the unaged Safaniya bitumen samples (Fig. 1). The fluorescence is attributed to conjugated aromatic ring systems that are characteristic of asphaltene molecules. Although the maltene phase of the bitumen may contain some conjugated aromatic structures, such as naphthalene derivatives, it is unlikely that any fluorescence from these molecules would be detected in the 515–545 nm range. The asphaltene structure is seen to form a dispersed phase in the continuous maltene matrix (Fig. 1), which is representative of the ‘sol’ structure expected of paving-grade bitumen. The compositions of the bitumens (Table 1) were used to calculate the colloidal instability index (CI), which is an indication of the dispersed nature of the bitumen. The CI values are relatively small, which is typical of paving-grade bitumen, and support the observation that the bitumens have a more dispersed structure than industrial-grade bitumen.

The size of the fluorescing particles is in the region of 2–7 µm, which is similar to the size of asphaltene aggregates observed by Loeber et al. (1996) using fluorescence microscopy and AFM. Rozeveld et al. (1997) observed that the asphaltene structure in an AC-10 bitumen consisted of interconnected ‘fibrils’ with a diameter in the order of 10 µm. These fibrils are

Table 1. Properties of the Safaniya and San Joaquin bitumens

<table>
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<th>Safaniya</th>
<th>Safaniya</th>
<th>San Joaquin</th>
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<td></td>
<td>180/200</td>
<td>80/100</td>
<td>AR4000</td>
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Table 1. Properties of the Safaniya and San Joaquin bitumens
thought to be composed of the resin and asphaltene phases of the bitumen. To verify that the fluorescence was in fact due to the asphaltene components of the bitumen, the unaged Safaniya 80/100 was separated into its asphaltene and maltene fractions. The CLSM image of the asphaltene fraction (Fig. 2) fluoresced uniformly, which indicates that the asphaltenes may be responsible for much of the observed fluorescence in the whole bitumen. The maltene fraction was examined under the CLSM in a similar manner to the undiluted bitumen. By comparing the maltene fraction (Fig. 3) with the whole bitumen (Fig. 1b) it is immediately obvious that the maltene fraction contains significantly fewer fluorescing regions than the whole 80/100 bitumen. There are two possible explanations for this. One explanation would be that the asphaltene fraction is responsible for the observed fluorescence and the separation of the bitumen into asphaltene and maltene fraction is not perfect, as fluorescing regions can still be observed in the maltene fraction. An alternative explanation is that the fluorescence remaining in the maltene fraction may be caused by molecules in this fraction that also exhibit fluorescence in the 515–545 nm region.

Fig. 1. CLSM image of Safaniya (a) 180/200 and (b) 80/100 bitumen. The light coloured flecks are thought to be asphaltenes aggregates dispersed in a darker maltene matrix. The asphaltene aggregates are typically 2–7 μm in size.

Fig. 2. Uniform fluorescence is observed in this CLSM image of asphaltenes precipitated from toluene.

The separation scheme used is based on the solubility of the asphaltene fraction in n-heptane, which is governed by its own equilibrium conditions. Consequently, it is possible that not all the asphaltene fraction precipitated from the n-heptane
be observed in bitumen using fluorescence microscopy. The asphaltenes, together with any adsorbed layer of resins, constitute the ‘solid’ dispersed phase of the bitumen as opposed to the amorphous, ‘liquid’ phase, which consists of non-crystalline saturates, aromatics and solvated resins. This provides a useful research tool for bitumen researchers who are interested in understanding how the structure of the dispersed phase affects its rheological properties.

Whereas the aromatic fraction fluoresces strongly, the resin and saturate fractions do not and may quench the fluorescence of the oily maltene phase. Consequently, the more amorphous maltene phase does not appear to fluoresce as strongly as the more crystalline asphaltene phase. We were also interested to see if changes to the structure of bitumen after ageing could be observed. The accelerated ageing regime employed is thought to be equivalent to approximately 5 years’ ageing of a chipseal binder in New Zealand (Bearsley, 2003). It is apparent from the fluorescent CLSM images of the aged 180/200 and 80/100 bitumen (Fig. 4) that the structure and fluorescence of the asphaltene phase do not alter radically upon oxidative ageing. This is in contrast to the work of Li & Wan (1995), who observed that freshly precipitated asphaltenes exhibit strong fluorescence, but after 48 h exposure to air the fluorescence emission had depleted to almost zero. It is possible that oxidation of the conjugated polyaromatic ring systems in the asphaltene fraction recovered by Li & Wan (1995) resulted in a fluorescence quenching mechanism. The mechanism of oxidation of asphaltenes as neat fractions may differ from that of asphaltenes in a bituminous matrix, leading to a difference in fluorescence properties observed by Li & Wan (1995). Solvent effects are known to affect both the intensity and the wavelength of the fluorescence emission (Guibault, 1990). Oxidation of the bitumen is known to alter the composition of the maltene phase, which may influence the fluorescence emission. That we would not observe any significant change in our sample was unexpected, especially as there was an increase in the colloidal instability index. However, the resolution of the micrographs makes a thorough analysis of the size and shape of the asphaltene particles difficult. Before drawing any conclusions from the ageing work, it may be useful to study the effect of oxidation on the fluorescent properties of the separated asphaltene and maltene phases.

A major advantage of using CLSM/fluorescence microscopy for observing the asphaltenes’ structure is that the bitumen requires minimal pretreatment and can be observed at ambient temperatures and pressures. The only pretreatment required is to heat the bitumen gently until it can flow before preparing microscope slides and allowing the bitumen to cool to ambient temperature. The structure of the dispersed asphaltene phase is dependent upon the temperature. It is believed that the evolution of the asphaltene structure with temperature is ‘due to a thermoreversible equilibrium’ (Lesueur et al., 1997). Consequently, the effect of mildly heating the bitumen for slide preparation is considered to be negligible because solution. We therefore consider that this is the most likely explanation for the presence of some remaining fluorescing regions in the maltene fraction. Therefore we believe CLSM is able to show the distribution of maltene and asphaltene components in bitumen.

To investigate further any other potential sources of fluorescence in the bitumen, the resin, aromatic and saturate fractions were examined using fluorescence microscopy. The aromatic fraction fluoresced strongly when observed under the microscope, but no fluorescence was observed for the resin and saturate fractions, in contrast to the work of Collins et al. (1991).

Popular models of bitumen structure suggest that the asphaltene micelles are coated in a layer of ‘peptising’ resin molecules (Lesueur et al., 1997). As it is generally accepted that the separation processes do not result in pure homogenous fractions, it is likely that the asphaltene fraction contains a proportion of the resin fraction adsorbed onto the surface of the particles. Although the resin fraction did not tend to fluoresce, it is not clear whether the resins that are adsorbed onto the asphaltene micelles contribute to the fluorescence or not. Because pure fractions cannot be obtained, it is not possible to determine whether they contribute to the fluorescence of the n-heptane insoluble, asphaltene fraction.

What is apparent is that the asphaltene fraction, as defined by the proportion of bitumen insoluble in n-heptane, can

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**Fig. 3.** Fluorescent CLSM image of the maltene phase of the Safaniya 80/100 bitumen after precipitation and filtration of the asphaltene fraction. Note the reduced number of fluorescing particles compared to Fig. 1, which indicates that the asphaltene phase is responsible for the observed fluorescence. The presence of fluorescing particles in the maltene phase suggests that the separation technique is not perfect.

the asphaltene structure returns to its original condition once the slides have cooled to ambient temperature. The relatively gentle and short heating regime is unlikely to have resulted in any significant ageing or oxidation of the samples.

With this technique, a visual comparison between bitumens that have different chemical compositions and structures can now be made. It is interesting to note that there does not appear to be much difference in the structure of the asphaltene phase of the 180/200 and 80/100 bitumens (Fig. 1). The two bitumens are from the same crude oil source but are different grades and have different rheological properties. Oxidation also does not appear to have much influence on the observed asphaltene structure. However, a difference was found in the structure of the asphaltene phase of bitumens obtained from different sources. It is interesting to note that although the colloidal instability index (which is an indicator of the colloidal structure) of the Safaniya and San Joaquin bitumens were similar, the structures differed. The AR4000 bitumen (Fig. 5) that was obtained from a San Joaquin crude appears to be more homogeneous than Safaniya bitumen. It is difficult to identify any dispersed fluorescent regions in the San Joaquin bitumen that could be identified as asphaltene aggregates. Obviously, the San Joaquin bitumen differs in both structure (Fig. 5) and chemical composition (Table 1) from the Safaniya bitumen. The San Joaquin bitumen also has a lower asphaltene content than the Safaniya bitumen and may lack molecular structures that fluoresce at 515–545 nm. It is possible that the structure may be so fine that it cannot be resolved adequately using the CLSM.

**Conclusions**

By combining CLSM and fluorescence microscopy it is possible to observe directly the asphaltene phase of the bitumen in situ.
Little pretreatment of the sample or preparation is required, which reduces the possibility of producing images that are not representative of the original material.

Fluorescence is influenced by the chemical nature of the bitumen, and this varies depending on the source of the bitumen. It is therefore probable that the optimal conditions for observing the fluorescence image of one bitumen will differ from that of bitumen from another source. The asphaltenes in Safaniya bitumen emit fluorescent light in the 515–545 nm region when irradiated with 488 nm wavelength light. The fluorescent properties of the asphaltenes were used to observe their dispersion and structure in the bitumen in situ.

The use of CLSM for observing the asphaltene phase of paving-grade bitumens in situ is a relatively new field and it is likely that improvements to the technique will allow higher quality images to be obtained. The images that we present are only two-dimensional cross-sections of the bitumen samples, whereas the construction of three-dimensional images is possible using CLSM. Such a three-dimensional analysis may provide more information on the size, shape and structure of the asphaltene phase. It may also be useful to quantify the structure of the bitumen phases using an image processing technique.

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References


