

Evaluating steady-state and time-resolved fluorescence as a tool to study the behavior of asphaltene in toluene†

Cite this: *Photochem. Photobiol. Sci.*, 2014, **13**, 917

Hui Ting Zhang,^a Rui Li,^a Zixin Yang,^a Cindy-Xing Yin,^b Murray R. Gray^{*b} and Cornelia Bohne^{*a}

A combination of steady-state fluorescence, fluorescence lifetime measurements and the determination of time-resolved emission spectra were employed to characterize asphaltene toluene solutions. Lifetime measurements were shown to be insensitive to the source of asphaltene or the alkane solvent from which asphaltene was precipitated. This insensitivity suggests that either the composition of Athabasca and Cold Lake asphaltene is very similar or that the fluorescence behavior is dominated by the same subset of fluorophores for the different samples. These results highlight the limitations in using fluorescence to characterize asphaltene solutions. Different dependencies were observed for the average lifetimes with the asphaltene concentration when measured at two different emission wavelengths (420 nm and 520 nm). This result suggests that different fluorophores underwent diverse interactions with other asphaltene molecules as the asphaltene concentration was raised, suggesting that models for asphaltene aggregation need to include molecular diversity.

Received 26th February 2014,
Accepted 31st March 2014

DOI: 10.1039/c4pp00069b

www.rsc.org/pps

Introduction

Asphaltene is defined as the fraction of crude oil that is insoluble in *n*-alkanes, such as heptane or pentane, but soluble in toluene. Asphaltene contains many different chemical species, some of which are fluorescent. The asphaltene fraction of crude oil is enriched in nitrogen, oxygen, vanadium and nickel. Asphaltene molecules can interact strongly leading to the formation of nano-structures with dimensions of 2 to 20 nm. These structures were detected by a variety of techniques, such as X-ray scattering,¹ membrane permeation,² Rayleigh scattering,³ and centrifugation.⁴ Fluorescence, due to its high sensitivity, has also been used to characterize asphaltenes. The emission spectra from asphaltene solutions are broad due to the presence of different fluorophores^{5–12} and shifts in the spectra and decreases in intensities at high asphaltene concentrations were related to aggregation of

asphaltenes.^{10,11,13} Different fluorophores in crude oils or asphaltene samples have different lifetimes and shorter lifetimes were observed when the concentrations of crude oil or asphaltenes were raised.^{7,12,14–17} These observations were attributed to energy transfer between separate asphaltene molecules or between asphaltene molecules within aggregates.

The objective of this work was to combine steady-state fluorescence, time-resolved fluorescence lifetime and time-resolved emission spectra (TRES) measurements to determine the sensitivity of fluorescence to the aggregation of asphaltene in toluene solutions. Two key experimental variables were: (i) a wide asphaltene concentration range from 0.1 mg L⁻¹ to 10 g L⁻¹ and (ii) the use of two different excitation (335 and 405 nm) and emission (420 and 520 nm) wavelengths. The wide concentration range employed spans the concentration range of 50 to 150 mg L⁻¹ for which asphaltene aggregation has been previously observed.^{13,18–20} However, the concentration range employed is not below the concentration range for which nanoaggregates are formed (<0.05 mg L⁻¹) as established by mass spectrometry experiments.²¹ Two excitation and emission wavelengths were used to probe if the concentration dependence on the lifetimes of excited states was dependent on the type of chromophore because different chromophores are excited at different wavelengths and different excited states can emit at different wavelengths. These experiments probe if different emissive asphaltene molecules undergo the same or different types of interactions that lead to

^aDepartment of Chemistry, University of Victoria, PO Box 3065, Victoria, BC, Canada V8W 3V6. E-mail: cornelia.bohne@gmail.com

^bDepartment of Chemical and Materials Engineering, University of Alberta, Edmonton, Alberta T6G 2G6, Canada. E-mail: gray@ualberta.ca

† Electronic supplementary information (ESI) available: Fluorescence of asphaltene at different excitation wavelengths, analysis of fluorescence decays, time-resolved asphaltene emission at 520 nm, average lifetime dependence with asphaltene concentration, fluorescence of pyrene in asphaltene, absorption of different asphaltene samples, average lifetime of AA-5 in toluene-heptane solutions. See DOI: 10.1039/c4pp00069b



changes in their photophysical properties when the concentration of asphaltene is raised. The focus of the experiments was to determine trends in lifetimes and time-resolved emission spectra as the concentration of asphaltene was raised. Detailed experiments were performed with Athabasca asphaltene precipitated from pentane (AA-5). For comparison purposes, selected experiments were performed for asphaltene from two different sources (Athabasca and Cold Lake) precipitated with either pentane or heptane, as well as with thermally cracked asphaltene.

Experimental section

Athabasca (AA) and Cold Lake (CL) asphaltenes were separated²² from bitumen by precipitation with pentane (AA-5, CL-5, Table 1) or heptane (AA-7, CL-7). The preparation of the thermally cracked samples (TC-AA-5 and TC-CL-5) was as follows: *ca.* 3 g of AA-5 or CL-5 was sealed in a microreactor and purged with N₂ and then H₂ (to 1.1 MPa). The microreactor was heated to 380 °C in a sand bath and shaken vertically for 40 min, followed by cooling and venting of the microreactor. The thermally cracked sample was dissolved in *ca.* 300 mL toluene, filtered through a 0.22 μm membrane and then concentrated and dried (yield of 92%). The apparent molecular weight measured by vapour pressure osmometry in *o*-dichlorobenzene decreased when the samples were thermally cracked (*e.g.* from *ca.* 1200 g mol⁻¹ for AA-5 to *ca.* 800 g mol⁻¹ for TC-AA-5).

Nitromethane (CH₃NO₂, 99+% Aldrich), tris-(2,2'-bipyridine) ruthenium(II) dichloride (Ru(bpy)₃²⁺, Sigma), heptane (Caledon, spectroscopic grade) and toluene (Caledon, spectroscopic grade) were used as received. Pyrene (99% Aldrich) was recrystallized once from ethanol and fluorescence lifetime measurements were used to check the pyrene's purity. A single lifetime (130 ns) was observed in aerated aqueous solutions.^{23,24}

Stock solutions of asphaltene (10 g L⁻¹) were prepared by dissolving the solid in toluene and solutions with lower concentrations were prepared by dilution. For experiments in toluene–heptane mixtures, a 1 g L⁻¹ stock solution of AA-5 was prepared in toluene and this stock solution was diluted in toluene–heptane to achieve a AA-5 concentration of 0.1 g L⁻¹. For the solution in heptane the solid was directly added to

heptane and the sample was sonicated for 40 min. AA-5 was not completely soluble at all heptane concentrations (*e.g.* 50% of AA-5 is soluble in pure heptane) and experiments were performed with the supernatant of these samples. Samples were measured on the day of preparation. All samples were then kept in a refrigerator and were warmed to room temperature for the measurements repeated 9 days after the initial sample preparation.

Pyrene (2–5 mM) and CH₃NO₂ (1 M) stock solutions were prepared in toluene and appropriate volumes were injected into asphaltene solutions. Aerated solutions were used because the objective of this work was the comparison of lifetimes and not the determination of absolute lifetime values. The exception was the experiments with pyrene where the lifetime of excited pyrene was lengthened by a factor of *ca.* 20 in deoxygenated solutions. Solutions containing pyrene and the CH₃NO₂ stock solution were deoxygenated by bubbling N₂ through the solution for 30 min. Samples were placed in 10 mm × 10 mm quartz cells and all experiments were performed at room temperature. CH₃NO₂ was injected into solutions containing pyrene using a gas tight syringe.

UV-Vis absorption spectra were measured with a Varian Cary 1 spectrometer. Steady-state fluorescence spectra were collected with a PTI QM2 fluorimeter using a Xe-arc lamp as the excitation source and a bandwidth of 3 nm for the excitation and emission monochromators. Raman scattering from the solvent was measured in a control experiment and this spectrum was subtracted from the emission spectra of asphaltenes.

Time-resolved fluorescence experiments were performed with an OB920 single photon counter (SPC) from Edinburgh Instruments. Samples were excited with either a light emitting diode (EPLD-330 at 335 nm) or with a laser diode (EPL-405 at 404 nm) supplied by Edinburgh Instruments. The bandwidth for the detection monochromator was set to 16 nm in order to maximize the collection rate. The instrument response function (IRF) was collected with an aqueous Ludox solution used to scatter the excitation light. Unless otherwise stated, data were accumulated until the number of counts in the channel with highest intensity reached 10 000.

Two types of geometrical arrangements between the emission and excitation optics were employed: (i) a 90-degree set-up between the excitation and emission optics was used for dilute samples. (ii) A front-face sample holder from Edinburgh Instruments was employed for measurements with highly absorbing samples (Fig. 1). The two geometrical arrangements were required because for highly absorbing samples the number of photons that reaches the centre of the cell with the 90-degree arrangement is negligible. For moderate concentrations of asphaltene the use of the front-face sample holder is also required because distorted spectra due to the re-absorption by asphaltene of the light emitted at short wavelengths were observed (see below). The front-face sample holder cannot be used with dilute solutions because photons are reflected towards the detector from the back wall of the cell leading to a change in the time-profile of the emission measured in time-resolved experiments.

Table 1 Properties of the Athabasca (AA) and Cold Lake (CL) asphaltenes²²

Asphaltene	Heptane insoluble content	Toluene insoluble content	V	Ni
	%		ppm	
CL-5	90	0.05	850	320
CL-7	100	0	790	280
AA-5	50	<0.05	580	230
AA-7	100	0	850	330



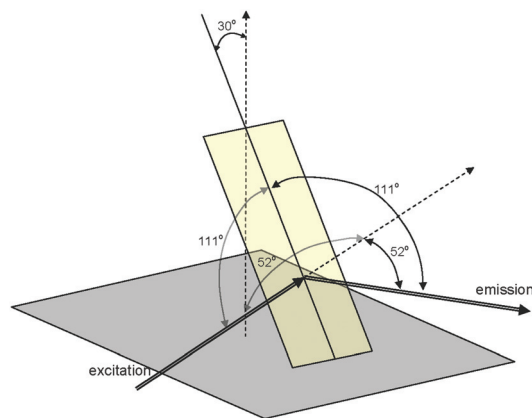


Fig. 1 Angles indicating the position of the sample placed in the front face sample holder. Diagram provided by Edinburgh Instruments Ltd.

The front-face sample holder was employed for samples that had an absorbance at the excitation wavelength equal or larger than 1.0. This threshold was determined by measuring the emission lifetime of Ru(bpy)₃²⁺ in aerated water ($\lambda_{\text{ex}} = 404$ nm) at various absorbance values. For absorbance values up to 0.8 to 1.0 the same lifetimes were recovered using both sample holders and the decays were mono-exponential. At absorbance values above 1 the decays were not mono-exponential for the sample with the 90-degree arrangement between the emission and excitation optics, while a mono-exponential decay was observed when using the front-face sample holder. Based on these results, experiments with asphaltene samples were performed with the front-face sample holder when the absorption of the excitation wavelength was close to 1 and higher.

The emission decays were fit to a sum of exponentials (eqn (1)) using the software from Edinburgh Instruments. A_i corresponds to the pre-exponential factor of species “ i ” and k_i corresponds to the decay rate constant of the excited state of “ i ”, which is equal to the inverse of its lifetime. The sum of all pre-exponential factors (A_i) is normalized to unity, and the magnitude of each pre-exponential factor is related to the abundance of that species. A re-convolution of the IRF to the calculated decay was performed to account for the IRF. A tail fit was employed, which does not use the re-convolution of the IRF, when the collection time window was long (≥ 2 μs).

$$I(t) = I_0 \sum_1^i A_i e^{-k_i t} \quad (1)$$

The goodness of the fit was judged by the χ^2 values recovered for the fit of the experimental data to eqn (1) and by the visual inspection of the residuals between the calculated and experimental values. Fits were considered adequate for χ^2 values between 0.9 and 1.2 and when a random distribution of residuals around zero was observed. The number of exponentials in the fit was increased until adequate fits were observed. At least five decays were collected for each sample. This average was considered the result for one independent experi-

ment. In most cases at least two independent experiments were performed where new solutions were prepared before each independent experiment.

Information from all fluorophores can be obtained by calculating the amplitude average lifetime ($\langle \tau \rangle$, eqn (2)), which is proportional to the emission intensity of the steady-state emission spectrum containing several fluorophores.^{25,26}

$$\langle \tau \rangle = \sum_1^i A_i \tau_i \quad (2)$$

TRES were obtained by collecting emission decays for a constant period of time at each detection wavelength. The spectra were constructed by integrating the intensities between defined time intervals for each one of the decays. For samples containing short- and long-lived emitting species, the spectra collected at short delays after excitation contain the composite spectra of all emitting species, while at long delays only the spectra for the long-lived excited states are measured.

Results

The absorption spectra for asphaltene solutions are broad (Fig. 2). The shape of the emission spectra depends on the excitation wavelengths employed (Fig. S1 in the ESI[†]) because at different wavelengths different subsets of chromophores or different concentration ratios of chromophores are excited. Changes in the shape of the emission spectra with the asphaltene concentration have been correlated to the aggregation of asphaltene.^{10,11,13} However, it is important to differentiate

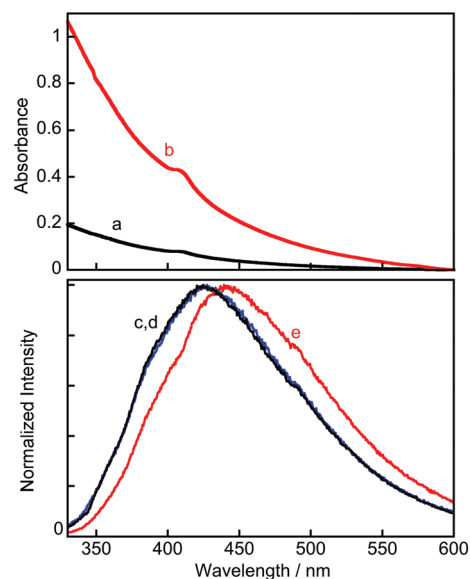


Fig. 2 Absorption spectra of AA-5 (top a: 10 mg L⁻¹, black; b: 50 mg L⁻¹, red) and normalized fluorescence spectra for asphaltene in toluene excited at 310 nm measured with the cell placed in a 90-degree arrangement between the excitation and emission optics (bottom c, black – 10 mg L⁻¹; e, red – 50 mg L⁻¹) and using the front-face sample holder (d, blue – 50 mg L⁻¹).



between an aggregate induced shift of the emission spectra and distortions due to artifacts, such as self-absorption. Such an artifact became apparent at relatively low asphaltene concentrations when the 90-degree arrangement between the excitation and emission optics was used (Fig. 2). The emission spectrum for the 50 mg L⁻¹ AA-5 solution was shifted to longer wavelengths when compared to the emission from a 10 mg L⁻¹ solution when the 90-degree arrangement sample holder was used. This effect was due to the high absorbance of the sample at shorter wavelengths ($A > 0.4$ for $\lambda < 400$ nm) and the shift disappeared when the 50 mg L⁻¹ asphaltene solution was measured using the front-face sample holder. This re-absorption effect was recognized in previous fluorescence studies with crude oils and asphaltenes where front-face sample holders were used or appropriate corrections were applied.^{9,10,14,27} However, in other studies this artifact was not recognized^{11,28,29} and, in these cases, conclusions on the aggregation of asphaltene based on spectral shifts are not warranted. The control experiment shown in Fig. 2 underscores the limitations of using steady-state emission spectra when studies are required for wide concentrations ranges where the absorption and emission spectra overlap, since artifacts were seen at fairly low asphaltene concentrations when using a 90-degree arrangement between the excitation and emission optics.

Analysis of changes for the steady-state emission spectra of complex mixtures is limited because the various fluorophores cannot be differentiated. Lifetime measurements introduce an additional experimental observable since excited states that emit at the same wavelengths can have different lifetimes. The decays for the emission of AA-5 were non-exponential indicating that more than one emissive species was present (Fig. 3). An emissive species in the context of this work is defined as either different fluorophores that have different intrinsic excited state lifetimes, or the same fluorophore present in different environments with different lifetimes in each environment. For example, in the latter case a fluorophore surrounded by inert solvent molecules could have a different lifetime from the same fluorophore located close to a quenching moiety of asphaltene.

The decay for the emission of AA-5 was adequately fit to the sum of three or four exponentials. Fits to a smaller number of exponentials were inadequate as indicated by the observation of high χ^2 values and of non-random residuals between the fit and the experimental data (Fig. S2 in the ESI†). It is important to note that in this study the interest was to establish trends for the lifetimes of the fluorophores at different asphaltene concentrations and the focus was not on determining the absolute values for the lifetimes. Fluorophores that have similar lifetimes will be grouped and detected as one lifetime. Therefore, the detection of one lifetime does not necessarily mean that there is only one species present with that particular lifetime. Studies with crude oils showed that average lifetimes for samples containing many fluorophores are dependent on the fitting model employed.³⁰ Therefore, comparisons of lifetimes obtained from fits of decays to the sum of

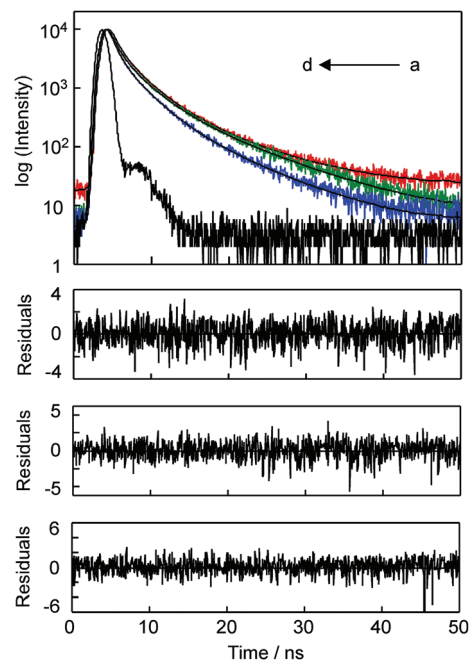


Fig. 3 Decay for the emission of asphaltene in toluene ($\lambda_{\text{ex}} = 335$ nm; $\lambda_{\text{em}} = 420$ nm) at different asphaltene concentrations (a, red – 0.1 mg L⁻¹; b, green – 50 mg L⁻¹; c, blue – 10 g L⁻¹; d, black – IRF). The fits of the data to a sum of four exponentials are shown in black and the residual plots are shown in the panels below the figure (top: 0.1 mg L⁻¹ – $\chi^2 = 1.083$; middle: 50 mg L⁻¹ – $\chi^2 = 0.995$ and bottom: 10 g L⁻¹ – $\chi^2 = 1.051$).

a different number of exponentials are not warranted. The decay for AA-5 in toluene at 420 nm excited at 335 nm could not be fit adequately to a sum of three exponentials for asphaltene concentrations of 100 mg L⁻¹ and 500 mg L⁻¹ (Table S1 in the ESI†). For this reason, the whole series of experiments at different asphaltene concentrations was fit to the sum of four exponentials (Table 2). The decays for the AA-5 emission at 520 nm when excited either at 335 nm or 405 nm were adequately fit to a sum of three exponentials (Tables S2 and S3 in the ESI†). Fits of the decays to a sum of four exponentials did not recover a fourth lifetime.

The lifetimes detected at 420 nm for the excitation of AA-5 at 335 nm were measured for a concentration range between 1 mg L⁻¹ and 10 g L⁻¹. Two different geometrical arrangements were required (see above). At intermediate asphaltene concentrations (10–50 mg L⁻¹) both sample holders could be used and the same trend for the lifetimes were observed despite the observation of slightly shorter average lifetimes when using the front-face sample holder (Table 2 and Fig. S3 in the ESI†). Up to a concentration of 50 mg L⁻¹ the average lifetimes for excited AA-5 were the same. At higher AA-5 concentrations the decays were faster (Fig. 3) and a shortening of all four lifetimes were observed (Table 2). In addition, at higher asphaltene concentrations, the A_1 value, which corresponds to the pre-exponential factor for the species with the shortest lifetime, increased at the expense of the other three A values. This result suggests that some fluorophores that had



Table 2 Lifetimes (τ), pre-exponential factors (A), and average lifetimes ($\langle\tau\rangle$) at different concentrations of AA-5 for the emission at 420 nm in toluene when excited at 335 nm

[AA-5]/mg L ⁻¹ ^a	τ_1 /ns (A_1)	τ_2 /ns (A_2)	τ_3 /ns (A_3)	τ_4 /ns (A_4)	$\langle\tau\rangle$ /ns
0.1 (2) ^b	0.41 ± 0.08 (0.44 ± 0.03)	1.4 ± 0.1 (0.39 ± 0.04)	3.7 ± 0.2 (0.14 ± 0.02)	9.7 ± 0.4 (0.025 ± 0.003)	1.48 ± 0.09
0.5 (2) ^b	0.42 ± 0.09 (0.40 ± 0.05)	1.3 ± 0.1 (0.41 ± 0.05)	3.5 ± 0.2 (0.16 ± 0.02)	9.2 ± 0.3 (0.025 ± 0.003)	1.5 ± 0.1
1 (6) ^b	0.48 ± 0.09 (0.44 ± 0.07)	1.4 ± 0.2 (0.39 ± 0.04)	3.8 ± 0.6 (0.14 ± 0.03)	10 ± 1 (0.026 ± 0.008)	1.51 ± 0.07
10 (9) ^b	0.50 ± 0.03 (0.47 ± 0.02)	1.45 ± 0.07 (0.38 ± 0.01)	3.9 ± 0.2 (0.13 ± 0.01)	9.5 ± 0.4 (0.024 ± 0.003)	1.50 ± 0.03
10 (2) ^c	0.39 ± 0.09 (0.52 ± 0.03)	1.4 ± 0.1 (0.36 ± 0.02)	4.2 ± 0.2 (0.10 ± 0.01)	10.2 ± 0.4 (0.017 ± 0.002)	1.31 ± 0.06
25 (2) ^b	0.50 ± 0.05 (0.49 ± 0.03)	1.5 ± 0.1 (0.36 ± 0.03)	4.0 ± 0.2 (0.12 ± 0.01)	9.9 ± 0.4 (0.023 ± 0.004)	1.51 ± 0.06
25 (2) ^c	0.37 ± 0.04 (0.53 ± 0.02)	1.4 ± 0.1 (0.34 ± 0.01)	4.0 ± 0.4 (0.11 ± 0.01)	10.1 ± 0.8 (0.018 ± 0.005)	1.28 ± 0.05
50 (4) ^b	0.41 ± 0.07 (0.47 ± 0.02)	1.4 ± 0.1 (0.37 ± 0.01)	3.7 ± 0.3 (0.13 ± 0.01)	9.3 ± 0.5 (0.025 ± 0.003)	1.41 ± 0.07
50 (4) ^c	0.31 ± 0.05 (0.55 ± 0.02)	1.30 ± 0.07 (0.32 ± 0.02)	3.6 ± 0.2 (0.11 ± 0.01)	9.2 ± 0.3 (0.022 ± 0.001)	1.17 ± 0.09
100 (4) ^c	0.21 ± 0.03 (0.60 ± 0.04)	1.23 ± 0.01 (0.29 ± 0.02)	3.6 ± 0.1 (0.10 ± 0.01)	9.2 ± 0.3 (0.018 ± 0.002)	1.0 ± 0.1
250 (2) ^c	0.22 ± 0.05 (0.61 ± 0.02)	1.2 ± 0.1 (0.27 ± 0.02)	3.5 ± 0.3 (0.10 ± 0.01)	9.1 ± 0.3 (0.018 ± 0.003)	0.97 ± 0.09
500 (2) ^c	0.25 ± 0.04 (0.60 ± 0.05)	1.24 ± 0.08 (0.28 ± 0.02)	3.6 ± 0.3 (0.10 ± 0.01)	9.4 ± 0.6 (0.017 ± 0.002)	1.04 ± 0.09
1000 (6) ^c	0.28 ± 0.05 (0.58 ± 0.05)	1.3 ± 0.1 (0.29 ± 0.03)	3.5 ± 0.2 (0.11 ± 0.02)	8.9 ± 0.3 (0.019 ± 0.003)	1.1 ± 0.1
2500 (2) ^c	0.17 ± 0.02 (0.67 ± 0.02)	1.12 ± 0.06 (0.23 ± 0.02)	3.2 ± 0.2 (0.09 ± 0.01)	8.3 ± 0.3 (0.017 ± 0.002)	0.78 ± 0.05
5000 (2) ^c	0.16 ± 0.02 (0.70 ± 0.02)	1.10 ± 0.06 (0.21 ± 0.02)	3.2 ± 0.2 (0.08 ± 0.01)	8.1 ± 0.4 (0.014 ± 0.002)	0.69 ± 0.06
10 000 (5) ^c	0.17 ± 0.01 (0.71 ± 0.02)	1.06 ± 0.01 (0.21 ± 0.01)	2.92 ± 0.09 (0.073 ± 0.003)	7.3 ± 0.2 (0.013 ± 0.002)	0.65 ± 0.03

^a Values in parenthesis correspond to the number of independent experiments. Errors for the average of two independent experiments correspond to average deviations or the propagation of individual error values, whichever is larger. Errors for more than two independent experiments correspond to standard deviations. ^b Experiments were performed with a 90-degree arrangement between the excitation and emission optics. ^c Experiments were performed using a front-face sample holder.

long lifetimes at low asphaltene concentrations have sufficiently short lifetimes at high asphaltene concentrations to be grouped with the short-lived fluorophores. This change in A values dictates that the analysis of individual lifetimes, as performed previously,¹⁴ is not warranted because some fluorophores are grouped into different lifetimes at different asphaltene concentrations. Therefore analysis of changes in lifetimes is warranted only for trends in the average lifetimes, limiting the type of information that can be obtained from lifetime measurements of these complex systems (see below).

The lifetimes for the emissive excited states in oils^{12,14,15,30} and asphaltenes^{7,16} depend on their composition and concentration. Our results are in qualitative agreement with these previous reports. However, the fit to a larger number of exponentials in our work revealed the presence of short-lived components. The fit to a larger number of exponentials also led to acceptable χ^2 values (≤ 1.3) compared to the values obtained with previous data analysis to the sum of two exponentials ($\chi^2 \sim 2$).¹⁴

Different mathematical models have been used to analyze the decays for the emission of asphaltene. Fluorescent species with similar lifetimes are grouped into one species when the emission decay is analyzed as a sum of exponentials. This approach is empirical since the number of fluorescent species in asphaltenes is not known and it is likely to be significantly higher than four. A different approach is to use a lifetime distribution analysis, which does not assume a number of exponentials. Analysis for the decay of the asphaltene emission at 511 nm when excited at 462 nm showed the presence of two populations of lifetimes with a significant distribution centred at 0.2–0.4 ns and 1.8–2.3 ns, and a shoulder around 10 ns.¹⁶ Although the lifetimes cannot be compared directly with our study because different excitation and emission wavelengths were used, our results are in qualitative agreement with the

lifetime distribution analysis in the sense that the decay can be fit to a finite number of lifetimes.

The amplitude average lifetime ($\langle\tau\rangle$, eqn (2)) is related to the emission intensity from all fluorophores. The dependencies of the average lifetimes with the AA-5 concentration were different for the emission at 420 nm and 520 nm (Fig. 4). For the emission at 420 nm, the values for $\langle\tau\rangle$, as well as the individual lifetimes and pre-exponential factors, did not change

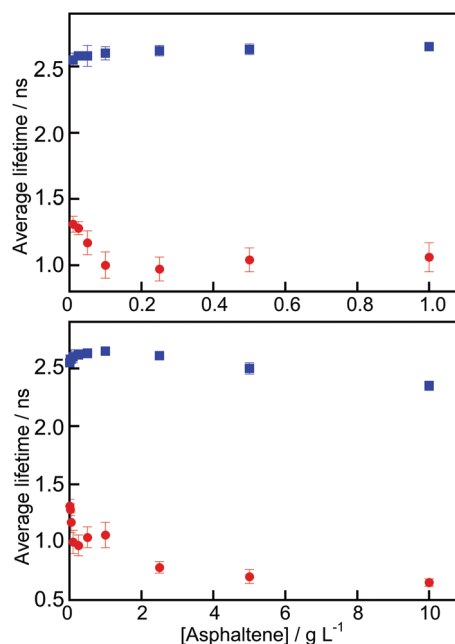


Fig. 4 Dependence with the asphaltene concentration of the average lifetime ($\lambda_{\text{ex}} = 335$ nm) for the asphaltene emission in toluene at 420 nm (red circles) and 520 nm (blue squares). All data were collected using a front-face geometry between the excitation and emission optics.



up to 50 mg L^{-1} of asphaltene (Table 2). A shortening of the average lifetimes was observed between 50 mg L^{-1} and 100 mg L^{-1} and the average lifetimes levelled off between 100 mg L^{-1} and 1 g L^{-1} . Most of the decrease observed in this concentration range was due to the shortening of τ_1 and increase in A_1 . A further decrease was observed at asphaltene concentrations higher than 1 g L^{-1} , where all lifetimes were shortened.

The average lifetimes for the emission of asphaltene at 520 nm were significantly longer by *ca.* 1.0 ns , than the $\langle\tau\rangle$ values measured for the emission at 420 nm (Fig. 4). The distribution of fluorophores between the different lifetimes was different at 420 nm and at 520 nm . At 420 nm the sum of the A values for the 3.7 ns and 9.7 ns lifetimes was 0.17 , while this sum at 520 nm for the 3.5 ns and 9.5 ns lifetimes was 0.45 , suggesting that a larger number of fluorophores with longer lifetimes emitted at 520 nm than at 420 nm . The dependence of the $\langle\tau\rangle$ values with the asphaltene concentration was different at 520 nm when compared to the dependence for the emission at 420 nm (Fig. 4). At 520 nm , no shortening of the average lifetime was observed around $50\text{--}100 \text{ mg L}^{-1}$ of asphaltene and a constant average lifetime was observed up to 1 g L^{-1} . Above 1 g L^{-1} the $\langle\tau\rangle$ values were shortened, which was due to a shortening of all individual lifetimes. The average lifetime for the emission at 520 nm was slightly shorter, by *ca.* 0.2 ns , when the sample was excited at 404 nm than when excited at 335 nm (Fig. S4 in the ESI†). However, the trends observed for $\langle\tau\rangle$ with the concentration of AA-5 were the same for samples excited at the two different wavelengths. Fig. 4 is the key experiment which shows that different fluorophores in asphaltene can display different behaviour with concentration changes, underscoring that the behaviour observed for one type of fluorophore (detected at one specific emission wavelength) is not representative of the behaviour of all asphaltene fluorophores.

Comparison of the TRES provides spectral information on the fluorophores with different lifetimes. The TRES in this study does not provide dynamic information on one fluorophore, but is related to the spectra of the subset of fluorophores with different lifetimes. At short delays, the TRES incorporates emission from all species, whereas at long delays the TRES is selective to species with long lifetimes because the contribution from the species with short lifetimes is decreased, or is negligible if the time window starts after the short-lived species decayed.

The TRES evolved over time with a shift of the emission maximum from 440 nm to 460 nm and the appearance of a shoulder between 500 nm and 550 nm . The TRES did not change after a delay of 15 ns . The dependence of the TRES at short delays ($0\text{--}5 \text{ ns}$) with the AA-5 concentration (1 mg L^{-1} to 10 g L^{-1}) provides information on all fluorophores (Fig. 5). The TRES did not change significantly when the concentration of AA-5 was raised, suggesting that the types of fluorophores did not change. For example, if a subset of specific fluorophores were not emissive or a new emissive species appeared as the concentration of AA-5 was varied then the spectra at short

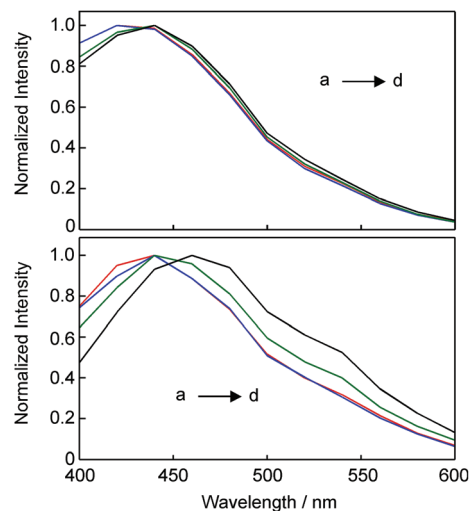


Fig. 5 Normalized TRES for asphaltene in toluene excited at 335 nm and either integrated between 0 and 5 ns (top panel) or 15 ns and 20 ns (bottom panel) for different asphaltene concentrations: 1 mg L^{-1} (a, red), 10 mg L^{-1} (b, blue), 1 g L^{-1} (c, green) and 10 g L^{-1} (d, black). The sample holder with a 90-degree arrangement between the excitation and detection optics was used for measurements with 1 mg L^{-1} and 10 mg L^{-1} asphaltene, while the front-face sample holder was used for samples containing 1 g L^{-1} and 10 g L^{-1} asphaltene.

delays would change because all emissive species were captured in the short time window.

The TRES at long delays ($15\text{--}20 \text{ ns}$) were red shifted as the concentration of AA-5 was raised. These TRES represent predominantly the two longest-lived species in asphaltene since the relative contribution of the two species with the shortest lifetime at 420 nm and the species with the shortest lifetime at 520 nm is decreased significantly. The change in the shape of the spectra at long delays shows that the contribution of fluorophores with long lifetimes changed as the concentration of asphaltene was raised. This result suggests that the fluorophores that emit at shorter wavelengths and had the higher excited state energy have shorter lifetimes when the AA-5 concentration was raised compared to the lifetimes in dilute solutions.

Pyrene was used as an external polyaromatic probe to determine the effect on the fluorescence behaviour of asphaltene when a probe with a known spectrum and lifetime is added to the solution. The fluorescence of pyrene has been extensively used to probe microheterogeneous systems^{31,32} because of its long singlet excited-state lifetime and the dependence of the shape of the steady-state emission spectrum with the solvent polarity. The lifetime of pyrene varies between 190 ns in de-aerated polar solvents to 650 ns in de-aerated non-polar solvents.³³ The property exploited for the current study was the long excited-state lifetime of pyrene, which can be differentiated from the lifetimes for the emission of asphaltene.

The emission of pyrene appears as a fine structure superimposed to the asphaltene emission (Fig. S5 in the ESI†). At low AA-5 concentrations the emission intensity from pyrene decreased because of the competitive absorption of photons



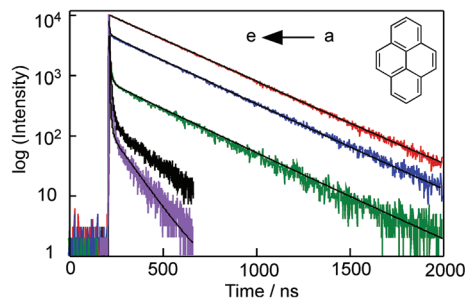


Fig. 6 Emission decays for pyrene (20 μM , $\lambda_{\text{ex}} = 335 \text{ nm}$, $\lambda_{\text{em}} = 391 \text{ nm}$) at various asphaltene concentrations: 0 mM (a, red), 10 mg L^{-1} (b, blue), 100 mg L^{-1} (c, green), 0.5 g L^{-1} (d, black) and 1 g L^{-1} (e, purple). The fits of the data to an exponential function (a) or the sum of four exponentials (b–e) are shown in black.

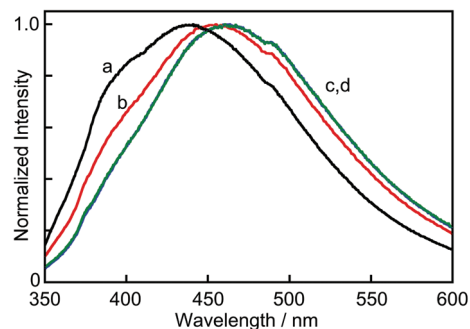


Fig. 7 Normalized steady-state emission spectra for the emission of 10 mg L^{-1} asphaltene solutions in toluene when excited at 335 nm: a (black) – Athabasca asphaltene precipitated from pentane (AA-5), b (red) – Cold Lake asphaltene precipitated from pentane (CL-5), c (blue) – Athabasca asphaltene precipitated from heptane (AA-7) and d (green) – Cold Lake asphaltene precipitated from heptane (CL-7). Note that the spectra for AA-7 and CL-7 overlay each other.

by pyrene and asphaltene. This effect precludes the analysis of the changes of the steady-state emission intensity of pyrene with the increase in the AA-5 concentration. The lifetime for the excited state of pyrene in deaerated toluene was much longer than the longest lifetime component from the asphaltene emission in a deaerated solution (*ca.* 300 ns for pyrene *vs.* 15 ns for 100 mg L^{-1} AA-5). Therefore, the dependence of the lifetimes for excited pyrene can be analyzed separately from the asphaltene emission when the solutions were deaerated. The pyrene emission in toluene follows a mono-exponential decay, while in the presence of asphaltene a fast decay from the asphaltene emission precedes the emission from pyrene (Fig. 6). The amplitude for the AA-5 emission increased with respect to the pyrene emission as the concentration of AA-5 was raised. In addition, a shortening of the pyrene lifetime was observed as the concentration of AA-5 was raised (Table S4 in the ESI†). At the highest asphaltene concentration of 1 g L^{-1} investigated, the pyrene lifetime was shortened to $121 \pm 1 \text{ ns}$. This shortening is due to quenching by asphaltene molecules. The quenching rate constant (k_{q}) was determined from the linear dependence between the observed rate constant (k_{obs}) with the quencher concentration, where k_0 is the decay rate constant of the excited state in the absence of quencher (eqn (3)). The rate constant for the quenching of the singlet excited state of pyrene by AA-5 was determined to be $(3.7 \pm 0.2) \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ by assuming an average asphaltene molecular weight of 750 g mol^{-1} (Fig. S6†).

$$k_{\text{obs}} = k_0 + k_{\text{q}}[\text{quencher}] \quad (3)$$

The quenching of excited pyrene by nitromethane was studied at various asphaltene concentrations. A shortening of the pyrene lifetime was observed and for all asphaltene concentrations investigated the quenching plots were linear (Fig. S7 in the ESI†). The quenching rate constants were constant (Table S5 in the ESI†) with a value of $(3.2 \pm 0.4) \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ in the absence of asphaltene and $(3.8 \pm 0.2) \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ in the presence of 1 g L^{-1} of asphaltene. This result suggests that the excited pyrene with a long lifetime resides in the homogeneous solution. A lower quenching rate constant

would be expected if pyrene was located inside the aggregate since access of nitromethane to the excited guest in the aggregate would be less likely than collisions between the excited state and quencher in solution.

The sensitivity of fluorescence lifetime measurements to the source of asphaltene was investigated by using Athabasca or Cold Lake asphaltene precipitated from either pentane (AA-5, CL-5) or heptane (AA-7, CL-7), and samples precipitated from pentane that underwent thermal cracking (TC-AA-5, TC-CL-5). The amount of heptane insoluble material in the pentane-precipitated fraction is 90% for CL-5 while it is 50% for AA-5. Vapour pressure osmometry measurements indicated that the apparent molecular weights of the thermally cracked samples were lower.

The emission spectra for AA-7 and CL-7 were the same; while blue shifted spectra were observed for AA-5 and CL-5 (Fig. 7). The largest blue shift was observed for AA-5, which corresponds to the sample that contains the largest portion of material that is insoluble in pentane but soluble in heptane. The changes observed for the emission spectra are not due to self-absorption at the shorter wavelengths since the absorption spectra for all samples were similar (Fig. S8 in the ESI†). Thermal cracking of the samples led to a blue shift of the emission spectra (Fig. S9 in the ESI†).

The decays for the emission of the various asphaltene samples excited at 335 nm were measured at 420 nm and 520 nm in order to provide a comparison with the experiments described for AA-5 above. The decays at both wavelengths were fit to the sum of four exponentials. Within experimental errors the average lifetimes were the same for all samples at each asphaltene concentration for the emission measured at 420 nm and 520 nm (Table 3). The trends observed for the individual lifetimes and pre-exponential factors were also similar for all samples. These results suggest that lifetime measurements are not very sensitive to changes in the composition of asphaltene despite changes being observed in the steady-state emission spectra.



Table 3 Average lifetimes ($\lambda_{\text{ex}} = 335 \text{ nm}$) for the emission at 420 nm or 520 nm of asphaltene from different sources and precipitated from either pentane or heptane

Asphaltene type ^a	$\lambda_{\text{em}}/\text{nm}$	Average lifetime/ns			
		Asphaltene concentration/g L ⁻¹			
		0.01	0.1	1.0	10
AA-5	420	1.39 ± 0.02	1.15 ± 0.09	1.1 ± 0.2	0.9 ± 0.1
AA-7	420	1.39 ± 0.05	1.04 ± 0.07	0.9 ± 0.2	0.5 ± 0.1
CL-5	420	1.55 ± 0.08	1.16 ± 0.04	1.1 ± 0.3	0.7 ± 0.1
CL-7	420	1.43 ± 0.07	1.0 ± 0.2	1.0 ± 0.1	0.6 ± 0.2
TC-AA-5	420	1.48 ± 0.07	1.07 ± 0.07	1.0 ± 0.3	0.7 ± 0.2
TC-CL-5	420	1.50 ± 0.07	1.12 ± 0.07	1.1 ± 0.2	0.7 ± 0.2
AA-5	520	2.53 ± 0.05	2.43 ± 0.05	2.3 ± 0.3	2.1 ± 0.2
AA-7	520	2.5 ± 0.1	2.26 ± 0.05	2.2 ± 0.2	1.6 ± 0.2
CL-5	520	2.50 ± 0.07	2.23 ± 0.06	2.3 ± 0.2	1.8 ± 0.2
CL-7	520	2.4 ± 0.2	2.1 ± 0.1	2.3 ± 0.2	1.8 ± 0.2
TC-AA-5	520	2.50 ± 0.07	2.3 ± 0.1	2.4 ± 0.1	2.1 ± 0.2
TC-CL-5	520	2.8 ± 0.2	2.2 ± 0.1	2.5 ± 0.1	1.9 ± 0.2

^a AA-5 and AA-7 corresponds to Athabasca asphaltene precipitated from pentane or heptane, respectively; CL-5 and CL-7 corresponds to Cold Lake asphaltene precipitated from pentane or heptane; TC-AA-5 corresponds to thermally cracked Athabasca asphaltene precipitated from pentane; TC-CL-5 corresponds to thermally cracked Cold Lake asphaltene precipitated from pentane. One independent experiment in which 3 or more decays were averaged. The errors correspond to standard deviations.

AA-5 was precipitated from pentane and this sample contained a 50% fraction that was not soluble in heptane. Lifetime measurements for the asphaltene emission (0.1 g L⁻¹) were performed for the supernatant of toluene solutions containing various fractions of heptane. The average lifetimes (Table S6 in the ESI†) recovered were the same (1.2 ± 0.1 ns). The lifetimes for the same solutions after 9 days were lengthened somewhat to 1.4 ± 0.1 ns for all solutions. These results suggest that the distribution of fluorophores between the toluene soluble material and material precipitated by heptane for an asphaltene sample is either the same or that changes in the composition are not sufficiently significant to be detectable by fluorescence lifetime measurements.

Discussion

The objective of this work was to evaluate the ability of time-resolved fluorescence techniques to characterize the aggregation of asphaltene. Fluorescence is a very useful technique to study complex systems because the properties of excited states can be very sensitive to the immediate environment around molecules. However, it is essential to realize that in a complex system containing many molecules, fluorophores will report about their immediate environment, but may not provide information about the material as a whole. Asphaltene is such a complex environment where a sub-set of molecules fluoresces.

The emissive components in asphaltene are mostly polyaromatic hydrocarbons some of which contain N or S hetero-

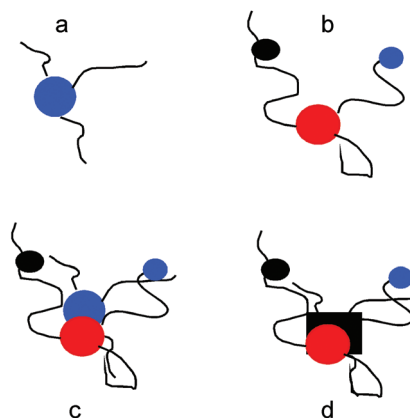


Fig. 8 Cartoon representation of the possible environments for fluorophores in individual asphaltene molecules and aggregates: (a) isolated fluorophore (blue); (b) different fluorophores (blue, red) and quencher moieties (black) in the same asphaltene molecule; (c) aggregation of asphaltene molecules where the fluorophores in the different molecules interact and (d) interaction of a molecule containing a fluorophore with another molecule containing a quencher.

atoms. Asphaltene also contains quenching moieties. Several possibilities exist for the arrangement of fluorophores and quenchers within individual asphaltene molecules, which provide distinct environments for the fluorophores (Fig. 8). The fluorophore can be isolated (Fig. 8a) or more than one fluorophore and quencher can be located in the same molecule (Fig. 8b). The fluorophores in the latter case can have the same photophysical properties as if they were independent and isolated, or can interact leading to quenching or formation of new emissive species. The formation of aggregates of chromophores is illustrative of the two modes of interaction, *i.e.* full quenching or formation of species with different emissive properties. H-aggregates where the chromophores are stacked are non-emissive, while J-aggregates where the chromophores are stacked in end-to-end or off-set arrangements can be emissive.^{34–38} The presence of quenchers when placed in proximity of the excited state can lead to static, *i.e.* immediate, quenching where the excited state is not detected at all in steady-state or time-resolved measurements. However, the presence of a quencher can also lead to a shortening of the excited state lifetime and a corresponding lower steady-state emission intensity. Such a scenario was observed in supra-molecular systems, as for example when pyrene was incorporated into a dendrocalixarene containing aromatic moieties.³⁹ This discussion shows that excited states of isolated asphaltene molecules can have different lifetimes either because the asphaltene molecules contain different fluorophores or the same fluorophore is in proximity of a second fluorophore or a quenching moiety. Therefore, lifetime measurements for dilute asphaltene solutions cannot be directly related to specific structures because the lifetimes cannot be compared to the lifetimes of model fluorophores.

Asphaltene molecules can interact either through the formation of aggregates containing several molecules or through



the collision between two molecules which then dissociate rapidly. Formation of aggregates and collisional encounters are both more prominent when the concentration of asphaltene is raised. The upper limit for a collisional process is set by the rate constant for the diffusion of two molecules. For this mechanism to operate, the concentration of quencher has to be sufficiently high for a collisional process to occur during the lifetime of the excited state. The lowest concentration for which a collisional quenching event occurs can be calculated using eqn (3) where the rate constant for diffusion of $1.1 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ in toluene³³ is equated to the quenching rate constant. The longest lifetime was 10 ns for the emission of AA-5 in toluene. A concentration of 0.48 mM of quencher would be required to shorten this lifetime to 9.5 ns. Collisional quenching can occur at AA-5 concentrations of 0.36 g L^{-1} or higher, assuming that all asphaltene molecules contain a quencher and that the average molecular weight of asphaltene is 750 g mol^{-1} .²⁰

Aggregate formation leads to the interaction of two or more asphaltene molecules. Fluorophores that are not in close proximity of other fluorophores or quenchers will have similar fluorescence properties to those observed for the isolated fluorophore. However, fluorophores can be placed in proximity of other fluorophores (Fig. 8c) or in the proximity of quenchers (Fig. 8d). Such interactions can lead to energy transfer processes with the population of species with lower excited state energies or can lead to quenching. In the latter case the quenching can be static or lead to shorter excited state lifetimes as mentioned above.

Steady-state emission spectra correspond to the emission of all chromophores taking into account the relative absorption coefficients at the excitation wavelength and the lifetime of each excited fluorophore. TRES segment the emission spectra based on the lifetimes of the fluorophores. At short delays, all fluorophores are detected. No changes were observed for the TRES at short delays when the AA-5 concentration was raised from dilute solutions, where AA-5 is not aggregated, to concentrations much higher than threshold for asphaltene aggregation (Fig. 5). All fluorophores are captured in the TRES at short delays and the fact that no changes were observed indicates that at all AA-5 concentrations the same population of fluorophores was excited and the excited states had a finite lifetime. The TRES at short delays would have changed if a subset of specific fluorophores were removed because of static quenching in the aggregates formed at high asphaltene concentrations. If significant static quenching occurred due to aggregation then all fluorophores would be involved in this process for the TRES not to change. Such a scenario is unlikely because of the molecular diversity of asphaltene and the requirement of close contact and defined alignment between chromophores for static quenching to occur. The examples mentioned above for the formation of emissive J-aggregates and supramolecular systems, where the excited guest is emissive despite the presence of quenching moieties in the host, supports the assignment that the fluorophores in asphaltene aggregates can be emissive and have finite lifetimes.

The TRES at long delay times show a marked red shift to longer wavelength for the solutions with high AA-5 concentrations. This result suggests that the fluorophores that are not present in the TRES at high AA-5 concentrations have a lifetime shorter than the time window for which the TRES was collected. These are fluorophores for which their excited state lifetimes were shortened because of the interaction with other fluorophores or with quenchers in the asphaltene aggregates. The observed red shift in the spectra shows that excited states with higher energies more readily undergo energy transfer to other fluorophores or to quenchers. In general, smaller molecules have higher excited state energies and emit at shorter wavelength for the same class of fluorophores. Therefore, as AA-5 aggregates the emission is predominantly related to the emission from larger aromatic molecules, which emit at longer wavelengths and have longer lifetimes.

A comparison of the steady-state emission spectra for asphaltenes from different sources and precipitated from different solvents is illustrative to determine the type of fluorophores present in these samples. The steady-state emission spectra of AA-7 and CL-7 were the same (Fig. 7) indicating that the fluorophores in these two sources of asphaltene are similar. The same conclusion was reached from the analysis of products formed in the controlled cracking of asphaltene.⁴⁰ The emission spectra were shifted to shorter wavelengths in the case of AA-5 and CL-5 indicating that the fractions that are pentane insoluble, but heptane soluble, have fluorophores with higher excited state energies than the fluorophores that are insoluble in heptane. Therefore, the fluorophores that are insoluble in pentane but soluble in heptane are likely to be smaller aromatic compounds. This interpretation is in line with the larger shift to shorter wavelengths observed for AA-5 when compared to CL-5, since AA-5 contains a larger fraction of pentane insoluble material that is soluble in heptane. The shift to shorter wavelengths observed for the emission spectra of thermally cracked samples is also consistent with the assignment of the emission at shorter wavelengths to smaller aromatic compounds, since the thermal treatment is likely to lead to the cleavage of small aromatic rings from larger asphaltene molecules.

In contrast to the emission spectra, the average lifetimes ($\langle \tau \rangle$) were shown *not* to be sensitive to the source of asphaltene or to the precipitation in different heptane–toluene solutions. It is important to note that the average lifetimes were also the same for thermally cracked samples showing that the fragmentation of asphaltene molecules did not have a significant effect on the lifetimes of the fluorophores. The insensitivity of the average fluorescence lifetime to the asphaltene composition is probably related to the relative large contribution of long-lived excited fluorophores, which may remain free in solution even when aggregates are formed. For example, when pyrene was added to an asphaltene solution its long lifetime was readily detectable (Fig. 6). Nitromethane quenching experiments showed that the pyrene with long lifetime was in the bulk toluene solution and was not protected by the aggregate. Despite an *A* value of less than 0.1 for pyrene (20 μM) in a



100 mg L⁻¹ (130 μM) AA-5 toluene solution, the average lifetime in the presence of pyrene was 21 ns compared to an average lifetime in the absence of pyrene of 1.4 ns. This example shows that a small amount of a long-lived species can dominate the average lifetime.

The dependence of the average lifetimes with the AA-5 concentration was different when the emission was measured at 420 nm or at 520 nm (Fig. 4), showing that different interactions between molecules of asphaltene occur as aggregates are formed. The shortening of lifetimes can be due to formation of aggregates or bimolecular quenching through the collision of two free asphaltene molecules. Both mechanisms have been previously proposed to be responsible for the shortening of lifetimes with increasing concentration of asphaltene or crude oils.^{7,12,14–17}

For the emission at 520 nm the average lifetime is constant up to a concentration of 1 g L⁻¹ of AA-5 with continuously shorter lifetimes being observed at higher asphaltene concentrations. The *A* values at this wavelength remain constant as the AA-5 concentration was raised while all three lifetimes were shortened (Tables S2 and S3 in the ESI†). These results are consistent with a bimolecular dynamic quenching mechanism since all fluorophores are affected equally and quenching only occurs at concentrations above 0.36 g L⁻¹ that is the lowest concentration for which bimolecular quenching would be expected. The higher threshold of 1 g L⁻¹ observed for AA-5 suggests that not all asphaltene molecules can act as quenchers, which is reasonable considering that asphaltene is known to contain aliphatic moieties in addition to aromatic ones,^{40–43} and not all polyaromatic compounds will have sufficiently low excitation energies to quench the excited states of fluorophores that emit at 520 nm.

The dependence of the average emission lifetimes with the AA-5 concentration at 420 nm shows a shortening of the lifetimes in the 50–100 mg L⁻¹ concentration range, followed by constant $\langle\tau\rangle$ values up to 1 g L⁻¹ and then a further shortening of the lifetimes at higher AA-5 concentrations. In addition, an increase was observed for the pre-exponential factor corresponding to the shortest lifetime (*A*₁, Table 2). The latter result suggests that the shortening of lifetimes for a subset of the fluorophores is more prominent than for other fluorophores. The shortening of the lifetimes in the 50–100 mg L⁻¹ concentration range cannot be due to a bimolecular processes since these reactions would have to occur with a rate constant higher than the theoretical maximum for a diffusion controlled process. Therefore, the shortening of the lifetimes in the 50–100 mg L⁻¹ concentration range is assigned to the formation of aggregates by some of the components of asphaltene that emit at 420 nm. The shortening of the lifetimes at AA-5 concentrations above 1 g L⁻¹ is likely due to bimolecular quenching as is the case for the emission at 520 nm but could also include a component due to further aggregation of asphaltene.

Other outcomes for the incorporation of asphaltene molecules in aggregates need to be considered. Sequential energy transfer could occur from fluorophores with high excited-state

energies to fluorophores with lower excited-state energies leading to a red shift in the spectra as observed in the TRES at long delays. However, the *A* values for the longer-lived species did not increase substantially (Tables S2 and S3 in the ESI†) as would be expected if sequential energy transfer occurred between fluorophores as aggregates are formed.

Formation of aggregates in solution raises the possibility of Rayleigh scattering, which would have the same time profile as the IRF. The intensity of Rayleigh scattering has a 1/λ⁴ dependence with the wavelength and its intensity increases with the increase in the size and number of particles.⁴⁴ The absorption of asphaltene solutions was shown to be a composite of molecular absorption and Rayleigh scattering.³ The size and/or number of aggregates are expected to increase as the concentration of asphaltene is raised and more scattering is expected at higher asphaltene concentrations and shorter wavelengths. The same TRES spectra were observed at short delays for all AA-5 concentrations indicating that the scattering contribution is not significant in the lifetime measurements.

The different lifetime dependencies with AA-5 concentration at 420 nm and 520 nm have consequences with respect to the models proposed for the structure of asphaltene. The “continental” model suggests asphaltene molecules correspond to polyaromatic cores surrounded by short alkyl chains,^{20,45} while in the “archipelago” model the molecules contain different molecular motifs that are linked by aliphatic moieties.^{42,43,46,47} No changes were observed for the lifetimes measured at 520 nm below 1 g L⁻¹ suggesting that the fluorophores emitting at this wavelength are not involved to any significant extent in the formation of aggregates, or if located in aggregates, the fluorophores do not interact with other fluorophores. In contrast, the changes in lifetimes measured at 420 nm suggest that these fluorophores interact consistent with aggregate formation. This differential behavior is inconsistent with the modified Yen model that assumes “continental” molecules and is based on the presence of a critical nano-aggregate concentration.²⁰ The concept of criticality cannot be applied to molecules that are different and are likely to aggregate under different conditions such as different concentration ranges. In addition, the Yen model assumes that π–π interactions are the driving force for aggregation, which would lead to the formation of non-emissive H-aggregates. Since at least some of the fluorophores in the aggregates are emissive different types of interactions are likely to occur and show that the Yen model is too simple. The different trends observed for the different fluorophores in asphaltene are consistent with the supramolecular model⁴⁶ where different types of interactions are proposed to be involved in the aggregation process.

The structures of the fluorophores in asphaltene are not known and different aromatic and heteroaromatic moieties are present.^{41–43} Statements as to trends in the emission spectra and lifetimes can be made at a qualitative level, but structural assignments are not possible since the spectra and lifetime values overlap for structurally different fluorophores. Therefore, previous attempts in correlating fluorescence with structural aspects of asphaltene, as summarized in support of the



modified Yen model,²⁰ may lead to empirical relationships but do not uncover mechanistic aspects on the structure of asphaltene components. No unified framework can be obtained from fluorescence studies. This conclusion is supported by the different concentration dependencies for the average lifetimes observed at different wavelengths in this work and the fact that different techniques observe aggregation behaviour in different concentration ranges depending on the sensitivity of the technique. While for the emission at 420 nm some asphaltene molecules aggregate at 50–100 mg L⁻¹, mass spectrometry experiments showed that aggregates are present at much lower concentrations.²¹

Fluorescence lifetime measurements are very valuable in systems with moderate complexity, such as supramolecular systems, where a finite number of fluorescent species (<5) are present and where the structure of the fluorophores and their photophysical properties are known. In these cases mechanistic information is obtained on the properties of the various environments for the fluorophores in the microheterogeneous system.^{31,32} This approach is not transferable to studies with asphaltene. Our lifetime studies provided qualitative, but limited, information because the fluorescence species could not be separated and only average lifetimes could be analysed. These average lifetimes were insensitive to the structural composition of the asphaltene samples, as shown for the comparison of the thermally cracked samples with the original samples. However, lifetime measurements were useful when probing for different experimental conditions when the same sample is studied, as for example for the changes in average lifetimes and TRES with the asphaltene concentration reported in this work.

Conclusions

The combined steady-state, TRES and time-resolved emission studies for asphaltene in toluene showed that the interpretation of fluorescence studies are useful at a qualitative level but cannot be correlated to structural aspects. Nevertheless, the dependencies of the average lifetimes measured at two different emission wavelengths showed that different fluorophores aggregate in different concentration ranges, suggesting that differential aggregation of asphaltene components occurs. The insensitivity of the average lifetimes to the source of asphaltene suggests that the fluorescence properties could be dominated by non-aggregated species with relatively long lifetimes. This situation parallels mass spectrometry experiments which were shown to be very sensitive to the asphaltene components that do not aggregate.²¹

Acknowledgements

We thank the Centre for Oil Sands Innovation at the University of Alberta for financial support.

Notes and references

- 1 R. Tanaka, E. Sato, J. E. Hunt, R. E. Winans, S. Sato and T. Takanohashi, *Energy Fuels*, 2004, **18**, 1118–1125.
- 2 G. P. Dechaine and M. R. Gray, *Energy Fuels*, 2011, **25**, 509–523.
- 3 M. Derakhshesh, M. R. Gray and G. P. Dechaine, *Energy Fuels*, 2013, **27**, 680–693.
- 4 F. Mostowfi, K. Indo, O. C. Mullins and R. McFarlane, *Energy Fuels*, 2009, **23**, 1194–1200.
- 5 T. Yokota, F. Scriven, D. S. Montgomery and O. P. Strausz, *Fuel*, 1986, **65**, 1142–1149.
- 6 O. C. Mullins, S. Mitra-Kirtley and Y. Zhu, *Appl. Spectrosc.*, 1992, **46**, 1405–1411.
- 7 C. Y. Ralston, S. Mitra-Kirtley and O. C. Mullins, *Energy Fuels*, 1996, **10**, 623–630.
- 8 H. Groenzin and O. C. Mullins, *J. Phys. Chem. A*, 1999, **103**, 11237–11245.
- 9 G. M. Pietraru and D. T. Cramb, *Langmuir*, 2003, **19**, 1026–1035.
- 10 F. C. Albuquerque, D. E. Nicodem and K. Rajagopal, *Appl. Spectrosc.*, 2003, **57**, 805–810.
- 11 S. Goncalves, J. Castillo, A. Fernández and J. Hung, *Fuel*, 2004, **83**, 1823–1828.
- 12 A. G. Ryder, *Appl. Spectrosc.*, 2004, **58**, 613–622.
- 13 D. Merino-Garcia and S. I. Andersen, *Pet. Sci. Technol.*, 2003, **21**, 507–525.
- 14 X. Wang and O. C. Mullins, *Appl. Spectrosc.*, 1994, **48**, 977–984.
- 15 A. G. Ryder, *Appl. Spectrosc.*, 2002, **56**, 107–116.
- 16 R. da Silva Souza, D. E. Nicodem, S. J. Garden and R. J. Correa, *Energy Fuels*, 2010, **24**, 1135–1138.
- 17 P. Owens and A. G. Ryder, *Energy Fuels*, 2011, **25**, 5022–5032.
- 18 S. Acevedo, M. A. Ranaudoa, J. C. Pereira, J. Castillo, A. Fernández, P. Pérez and M. Caetano, *Fuel*, 1999, **78**, 997–1003.
- 19 I. N. Evdokimov, N. Y. Eliseev and B. R. Ashmetov, *Fuel*, 2003, **82**, 817–823.
- 20 O. C. Mullins, *Energy Fuels*, 2010, **24**, 2179–2207.
- 21 A. M. McKenna, L. J. Donald, J. E. Fitzsimmons, P. Juyal, V. Spicer, K. G. Standing, A. G. Marshall and R. P. Rodgers, *Energy Fuels*, 2013, **27**, 1246–1256.
- 22 C.-X. Yin, J. M. Stryker and M. R. Gray, *Energy Fuels*, 2009, **23**, 2600–2605.
- 23 W. Xu, J. N. Demas, B. A. DeGraff and M. Whaley, *J. Phys. Chem.*, 1993, **97**, 6546–6554.
- 24 H. Yang and C. Bohne, *J. Phys. Chem.*, 1996, **100**, 14533–14539.
- 25 A. Sillen and Y. Engelborghs, *Photochem. Photobiol.*, 1998, **67**, 475–486.
- 26 S. E. Webber, *Photochem. Photobiol.*, 1997, **65**, 33–38.
- 27 M. H. Ghatee, B. Hemmateenejad, T. Sedghamiz, T. Khosousi, S. Ayatollahi, O. Seiedi and J. S. Amin, *Energy Fuels*, 2012, **26**, 5663–5671.



- 28 A. K. Ghosh, S. K. Srivastava and S. Bagchi, *Fuel*, 2007, **86**, 2528–2534.
- 29 M. Daaou, D. Bendedouchm, A. Modarressi and M. Rogalski, *Energy Fuels*, 2012, **26**, 5672–5678.
- 30 P. Owens, A. G. Ryder and N. J. F. Blamey, *J. Fluoresc.*, 2008, **18**, 997–1006.
- 31 K. Kalyanasundaram, *Photochemistry in Microheterogeneous Systems*, Academic Press, Orlando, 1987.
- 32 M. H. Kleinman and C. Bohne, in *Molecular and Supramolecular Photochemistry*, ed. V. Ramamurthy and K. S. Schanze, Marcel Dekker Inc., New York, 1997, pp. 391–466.
- 33 M. Montalti, A. Credi, L. Prodi and M. T. Gandolfi, *Handbook of Photochemistry*, CRC Press, Boca Raton, 2006.
- 34 A. Chowdhury, S. Wachsmann-Hogiu, P. R. Bangal, I. Raheem and L. A. Peteanu, *J. Phys. Chem. B*, 2001, **105**, 12196–12201.
- 35 M. Kasha, *Radiat. Res.*, 1963, **20**, 55–71.
- 36 F. C. Spano, *Acc. Chem. Res.*, 2010, **43**, 429–439.
- 37 D. G. Whitten, *Acc. Chem. Res.*, 1993, **26**, 502–509.
- 38 K. Hannah and B. A. Armitage, *Acc. Chem. Res.*, 2004, **37**, 845–853.
- 39 C. Yihwa, M. Kellerman, M. Becherer, A. Hirsch and C. Bohne, *Photochem. Photobiol. Sci.*, 2007, **6**, 525–531.
- 40 R. I. Rueda-Velásquez, H. Freund, K. Qian, W. N. Olmstead and M. G. Gray, *Energy Fuels*, 2013, **27**, 1817–1829.
- 41 O. P. Strausz, T. W. Mojelsky, F. Faraji, E. M. Lown and P. Peng, *Energy Fuels*, 1999, **13**, 207–227.
- 42 O. P. Strausz, T. W. Mojelsky and E. M. Lown, *Fuel*, 1992, **71**, 1355–1363.
- 43 J. M. Sheremata, M. R. Gray, H. D. Dettman and W. C. McCaffrey, *Energy Fuels*, 2004, **18**, 1377–1384.
- 44 J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, Springer, New York, 2006.
- 45 J. P. Dickie and T. F. Yen, *Anal. Chem.*, 1967, **39**, 1847–1852.
- 46 M. R. Gray, R. R. Tykwinski, J. M. Stryker and X. Tan, *Energy Fuels*, 2011, **25**, 3125–3134.
- 47 A. Karimi, K. Qian, W. N. Olmstead, H. Freund, C. Yung and M. R. Gray, *Energy Fuels*, 2011, **25**, 3581–3589.

