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Fractionation of a wood tar pitch by planar chromatography for the characterisation of large molecular mass materials

M.J. Lazaro[a], M. Domin[b], A.A. Herod[a,*], R. Kandiyoti[a]

[a]*Department of Chemical Engineering and Chemical Technology, Imperial College, University of London, London SW7 2BY, UK*
[b]*Department of Pharmaceutical and Biological Chemistry, School of Pharmacy, Brunswick Square, London WC1N 1AX, UK*

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Abstract

A commercial tar pitch derived from pine wood – Massen Pine (*Pinus Massonia*) and sold as Stockholm tar has been fractionated by planar chromatography with examination of the fractions by size exclusion chromatography in NMP eluent, by UV-fluorescence and by matrix assisted laser desorption mass spectrometry. The relatively small molecules, mobile in planar chromatography, are shown to be non-polar. Large molecules were found in each fraction, corresponding in SEC elution times up to polystyrenes of molecular mass of at least 1.8 million. Size exclusion chromatography profiles by UV light absorbance showed differences in relative absorbance of different wavelengths for large and small molecules, implying differences in structures. MALDI mass spectra indicated molecules of mass of several thousand mass units with the upper limit of mass not defined. Planar chromatography provides a fast, cheap method of isolating large molecular mass fractions of this biomass tar.

Keywords: Tar

1. Introduction

In earlier work [1–6] we have investigated molecular mass (MM) distributions of coal liquefaction extracts, pyrolysis tars, coal tar pitch, petroleum residues, a naphthalene mesophase pitch and a fullerite mixture mainly by matrix assisted laser desorption ionisation (MALDI)-mass spectrometry and size exclusion chromatography (SEC) using 1-methyl-2-pyrrolidinone (NMP) as eluent. This work has led to the identification of materials of very large molecular mass, ranging in mass from several thousand mass units up to in excess of 100 000 u. Clearer observations of high molecular mass materials in coal derived liquids [2,7], petroleum residues [5] and a napthalene mesophase pitch [6] were made possible by fractionation of the samples, using both planar chromatography and separation by solvent solubility. Fractions separated from coal-derived materials and petroleum vacuum residues [5] by planar chromatography showed different behaviour in UV-fluorescence spectra.

Biomass derived liquids normally contain a high proportion of oxygenates. Product evaluation work has been dominated by extensive use of GC–MS [8–12], with no attempt to investigate the presence

*Corresponding author. Tel.: +171-589-5111; fax: +171-594-5604.
E-mail address: a.herod@ic.ac.uk (A.A. Herod)

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of larger molecular masses. In the case of highly oxygenated – i.e. highly polar compounds – or of polynuclear aromatic hydrocarbons, molecules with masses above 300 u are not normally observed by GC or GC–MS. A larger proportion of components in biomass derived liquids simply do not go through a chromatographic column, because of their high molecular masses (MM) and/or high polarities. Data based on SEC and MALDI of biomass derived tars presented in a previous paper [13] confirm that significant proportions of the samples have MMs well above the range available to GC–MS or heated probe-MS.

Several samples of pitch recovered from the wreck of the flagship *Mary Rose* [14,15] have been examined and compared with a Stockholm tar sample. GC and GC–MS analysis of the components of the *Mary Rose* pitches eluting in hexane, toluene and dichloromethane, and those obtained by esterification of the acid fraction, revealed in every case relatively simple mixtures of compounds. Similarities between the *Mary Rose* samples and Stockholm tar (good-quality pine tar pitch obtained by the destructive distillation of *Pinus Sylvestris*) have been found by the techniques used, GC–MS and NMR. The sample of Stockholm tar used in the present work was from a different species of pine, *Pinus Massonia*.

Earlier work with this pine wood tar pitch [16] indicated the presence of a very wide range of molecular masses by SEC and although the pitch, with an oxygen content of 5%, is not typical of tars derived from biomass in general [13], it does represent the current product of a process which has been in use for many centuries. In this paper, a pine wood tar pitch commercially available as Stockholm tar but not necessarily identical to that used elsewhere [14,15] has been fractionated by planar chromatography using two different solvent systems to develop the plates: (i) pyridine followed by acetonitrile and (ii) THF followed by toluene. The whole tar and the fractions have been characterized by size exclusion chromatography (SEC), UV-fluorescence spectroscopy (UV-F) and matrix assisted laser desorption ionization mass spectrometry (MALDI-MS) with the object of establishing the presence of large molecular mass materials, for comparison with similar data for coal tars and petroleum vacuum residues.

2. Experimental

2.1. Sample

A pine wood tar commercially available as Stockholm tar was studied, supplied by Battle Hayward and Bower, Lincoln, UK, but imported by White Sea and Baltic of Leeds, UK. This tar was prepared by the destructive distillation of wood of the Massen Pine (*Pinus Massonia*) in South China to produce a crude tar liquid which is imported to UK, distilled to remove water, volatile components and induce polymerisation and produce tar of different viscosities before sale as an equine antiseptic treatment. The material used here corresponds to a thick, high-viscosity product, sold as Stockholm tar. The oxygen content of this tar was about 5%, whereas that of the *Mary Rose* comparison [14,15] was approximately 11%.

2.2. Planar chromatography

Procedures for the fractionation of samples by planar chromatography have been described elsewhere [2,7,17]. Briefly, chromatography was performed on 10×20 cm Whatman K6 silica gel plates developed with two solvent systems: (i) pyridine followed by acetonitrile and (ii) THF followed by toluene. Before use, the plates were washed in the more polar solvent, pyridine or THF, to remove contaminants from the coating materials, and then dried. Whereas 1-methyl-2-pyrrolidinone (NMP) appears to dissolve the bio-mass tar completely, it cannot be readily evaporated from the plate, because its boiling point is over 200°C at atmospheric pressure. Samples were, therefore, applied to the plates as partially dissolved slurries in pyridine. A narrow band of the slurry was applied at the origin, along a 20 cm side, by multiple spotting; the plate was dried in air before development.

Development tanks were equilibrated for 30 min to saturate the vapor phase before insertion of the plates. The first development in the more polar solvent proceeded for 2–3 cm; the plates were removed from the tank and dried before insertion into the second solvent for development for a further 2 or 3 cm beyond the first solvent front.

After the final drying, bands of silica were scraped from the plates; these represented: (i) material immobile in both solvents, (ii) material at the first solvent front (not mobile in the second solvent) and (iii) material mobile in both solvents and near to the second solvent front. Recovery of the wood tar fractions from the silica was achieved by dissolution at room temperature in 1-methyl-2-pyrrolidinone (NMP) assisted by ultrasonic agitation. The recovered fractions were semi-preparative in nature rather than analytical; no attempt was made to recover quantitative fractions.

2.3. UV-fluorescence spectroscopy

Descriptions of the procedure for acquiring UV-fluorescence spectra have been given elsewhere [18–20]. The Perkin-Elmer LS50 luminescence spectrometer was set to scan at 240 nm min^{-1} with a slit width of 2.5 mm: synchronous spectra were acquired at a constant wavelength difference of 20 nm. A quartz cell with 1 cm path length was used. The spectrometer featured automatic correction for changes in source intensity as a function of wavelength. Acquisition of emission, excitation and synchronous spectra of the samples were performed in NMP. Because of uncertainties in the mass of wood tar derived materials in fractions recovered from the silica gel, the spectra have been presented in peak-normalized mode. Solutions of sample fractions were diluted with NMP to avoid self-absorption effects [18].

2.4. Size exclusion chromatography

SEC using NMP as solvent was carried out using a 3-μm Mixed-E polystyrene–polydivinylbenzene column (Polymer Laboratories Ltd.) with a flow rate of 0.45 ml min^{-1} at a column temperature of 85°C into two different detectors in series: a variable wavelength Perkin-Elmer LC290 UV at 450 nm and a diode array detector set at 280 nm, 300 nm, 350 nm and 370 nm. The same sample injection was used to record all the profiles in order to compare the spectra at the different wavelengths. The Perkin-Elmer LC290 UV detector has been used previously [1–3,7,21]. Data were collected simultaneously from both detectors into a computer. The elution volume corresponding to the exclusion limit of the column was approximately 5.8 ml (corresponding to a polystyrene molar mass between 30 and 40 000 u); calibration curves have been shown previously [6]. The exclusion limit is defined by the polystyrene standard eluting at the upper mass end of the linear relation between log 10 molecular mass and elution volume; higher mass standards elute with some separation, but with a different relation to elution volume.

2.5. MALDI-MS spectrometry

A Fisons VG-TOFSPEC instrument was used in linear mode, with a nitrogen laser with a VAX 4000-based data system with OPUS software. Several matrices have been tried to generate spectra from the tar; 2,5-dihydroxybenzoic acid (2,5 DHB), 2-mercaptobenzothiazole (MBT), dimethoxy-4-hydroxycinnamic acid (sinapinic acid), α-cyano-3-hydroxycinnamic acid (α-cyano), in the presence and absence of silver trifluoroacetate cationizating agent. Sample and matrix solutions in NMP were deposited onto the target by vacuum drying; deposition of NMP alone produced no signal above m/z 200. An ion accelerating voltage of 28 kV was used with maximum laser power and approximately 50 spectra were summed in each case.

3. Results and discussion

3.1. Planar chromatography

Because of the relative simplicity of the scheme used in this study, details of the planar chromatography separation in terms of relative retention factors (R_F) of the fractions have not been given. The object was to produce a type separation on the basis of solution mobility rather than on a series of separated spots. Fractions were collected as either immobile in both solvents, mobile in the more polar and immobile in the less polar solvent, or mobile in both solvents. Visual inspection of the developed plates indicated that there was relatively little of the sample left at the origin. The colours of the bands were

different, with the most mobile fractions appearing yellow or orange, the fractions mobile in one solvent being brown and the immobile fractions black. The fractions produced in this work have been designated as A, B and C (for the pyridine–acetonitrile solvent system) and D, E and F (for the THF–toluene solvent system), with increasing mobility in planar chromatography, in accord with previous work on a coal tar pitch [2,5,7].

3.2. Size exclusion chromatography

In terms of polystyrene standards, the exclusion limit of the present column is between 30 000 and 40 000 u. This range represents the upper limit of the linearity in terms of the relationship between log-(molecular mass) and elution volume, for the particular column. Above 40 000 u, the relationship becomes non-linear: polystyrene standards of nominal mass 87 000, 194 000, 460 000, and 1 850 000 u eluted from the column used for UV-A at elution times of 12.2, 12.0, 11.6, and 11.0 min, respectively. A calibration curve for this column has been shown previously [6]. The planar chromatographic separation indicated that much of the retained material, mobile in toluene, may be of low polarity and therefore it is highly probable that the material of the wood tar which was excluded from the column, shown in Figs. 1 and 2, corresponds to very high MMs.

Fig. 1 shows the size exclusion profiles of the Stockholm tar at four wavelengths in NMP solvent. The peak corresponding to small molecules, between 15 and 24 min, has the major intensity of absorbance at 300 nm, with absorbance at 280 nm greater than that at 350 nm. In the excluded peak corresponding to the largest molecules, between 10 and 13 min, absorbance at 300 nm is still the most intense, but that at 350 is now more intense than at 280 nm. This indicates that the aromatic systems in the excluded material are different from and greater than those in the retained material.

Fig. 2A–F shows profiles at four wavelengths for the respective fractions A to F derived from the planar chromatographic separation. The profiles for fractions A (immobile in pyridine and acetonitrile) and D (immobile in THF and toluene) depart from baseline before 10 min elution time, implying that their size is equivalent to polystyrenes of molar mass 2 million approximately. All of the fractions show evidence of material near the exclusion limit of the column (although the peak at about 12 min in fraction F is small) as well as material within the operating range, implying a wide range of molecular sizes, as defined by the planar chromatographic separation. The proportions of the excluded material

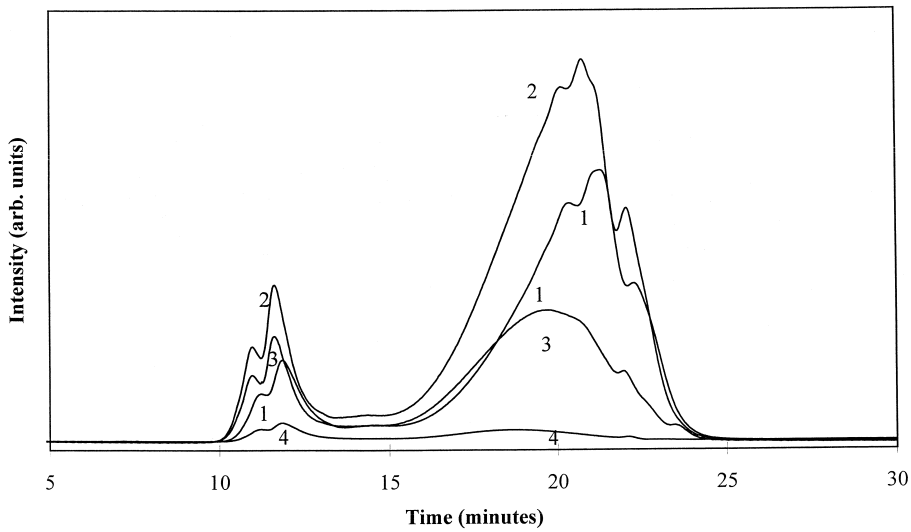

Fig. 1. SEC profiles of Stockholm tar at four wavelengths: 1–280 nm; 2–300 nm; 3–350 nm; 4–450 nm.

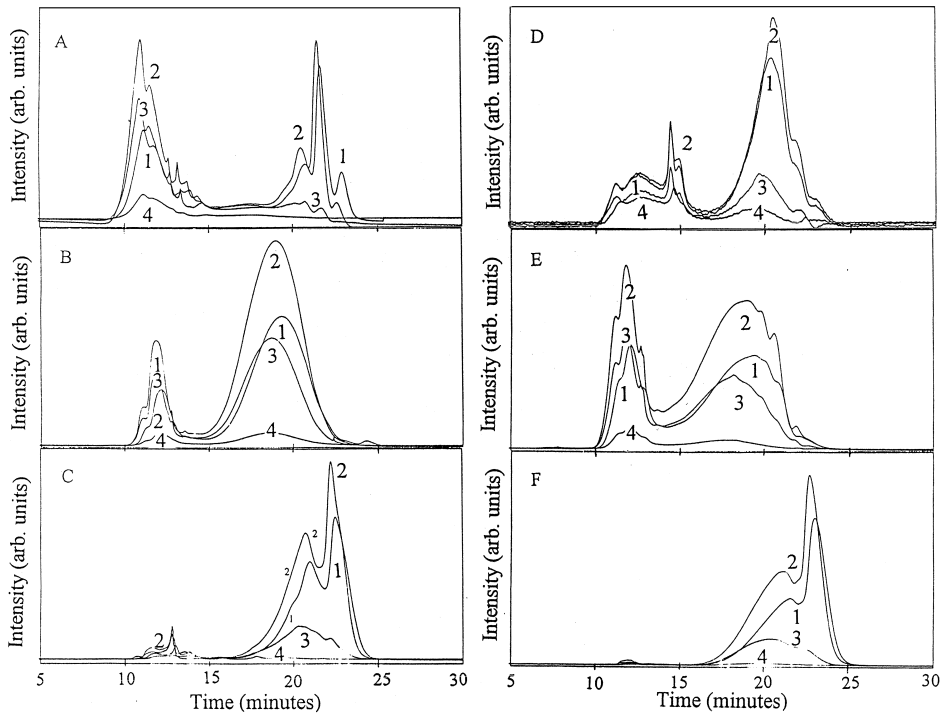

Fig. 2. SEC profiles of TLC fractions, A) fraction A immobile in pyridine–acetonitrile, B) fraction B mobile in pyridine, C) fraction C mobile in acetonitrile, D) fraction D immobile in THF/toluene, E) fraction E mobile in THF, F) fraction F mobile in toluene. Wavelengths: 1–280 nm; 2–300 nm; 3–350 nm; 4–450 nm.

to retained material change with solvent polarity in planar chromatography, with less excluded in the less polar solvents (toluene and acetonitrile) than in the more polar solvents (THF and pyridine). In each case, the molar size range of the fractions increases with increasing immobility, with an increasing proportion of excluded material, indicating that the planar chromatography separation is a function of increasing molecular size. The region between the excluded peaks and the retained peaks did not reach baseline in any of the SEC profiles, indicating that there was a continuum of molecular size, from large to small. The relatively smaller peak of excluded material in the SEC profile of the whole Stockholm tar points to the masking effect of the greater concentration of the smaller MM and more mobile material, underlining the utility of the planar chromatographic separation. In each part of Fig. 2A–F, the absorbance at 300 nm is the most intense, with absorbance at 280 nm greater than at 350 nm for the retained peak, while for the excluded peak absorbance at 350 nm is greater than at 280 nm, as with the whole tar in Fig. 1. Comparison of profiles for fractions B and C, and E and F, indicates that the toluene–THF separation was more complete than that with pyridine–acetonitrile; the proportions of excluded peak to retained peak are more extreme for toluene–THF than for pyridine–acetonitrile. This completeness of separation is confirmed by the UV-fluorescence results (below) which showed that the THF–toluene solvent system produced a more complete separation than pyridine–acetonitrile, indicating that the most mobile material is probably non-polar. For both solvent systems, the immobile fractions A and D gave relatively weak absorbance profiles in SEC because their concentrations were low. The significance of all the peaks is not understood, especially the peaks near 15 min in Fig. 2D; we note however the presence of a small unresolved peak in Fig. 1 at 14–15 min which may be equivalent.

Absorbance at different wavelengths of UV light is a function of the different aromatic cluster sizes in

the sample molecules and changes in the relative intensities of absorbance of the fractions indicate structural changes with changing molecular size. Because the measurements with all the wavelengths have been carried out using the same injected solution, additional information can be obtained by comparing the four wavelengths in the same figure for each fraction without normalisation. At 450 nm, the larger aromatic clusters are dominant, whereas at 280 nm more information about the smaller aromatic clusters and low molecular mass material can be obtained. In all of the fractions and the whole sample the absorbance at 300 nm is the most intense, with absorbance at 280 and 350 nm varying in relative intensity in the excluded or retained peaks. At 450 nm, the absorbance may be expected to correspond to only the very large aromatic clusters and the most polar molecules. The absorbance profile at 450 nm in Fraction F is very small, with only a slight intensity in Fraction C. The absorbance profiles of the partly mobile and immobile fractions A, B, D and E all show greater intensity at 450 nm than those for the very mobile fractions, with the greatest relative intensity in the excluded peak rather than the retained peak. These shifts of relative intensity reinforce the view that the excluded peak corresponds to molecular structures which are different from those of the retained peak and correspond to larger molecular sizes and aromatic systems.

3.3. UV-fluorescence spectroscopy

Fig. 3 presents synchronous UV-fluorescence spectra of Stockholm tar and its fractions B (mobile in pyridine) and C (mobile in pyridine and acetonitrile). Synchronous spectra of the tar and the analogous fractions E (mobile in THF) and F (mobile in THF and toluene) are presented in Fig. 4. The profiles are height-normalised. UV-F spectra of fractions A (immobile in both pyridine and acetonitrile) and D (immobile in THF and toluene) can not be shown because they did not fluoresce at all indicating the presence of very high molecular mass material.

The fractions mobile in both solvents showed relatively strong fluorescence intensities, the position of the peaks at lower wavelengths suggesting the presence of relatively smaller polynuclear aromatic ring systems and probably also the presence of lower molecular mass material. Similarly, fractions B and E (mobile in one solvent) both gave less intense fluorescence than fractions C and F (mobile in both solvents). The fluorescence intensities fall with increasing immobility, as in the case of other samples studied before [2,5,7,22], where fluorescence quan-

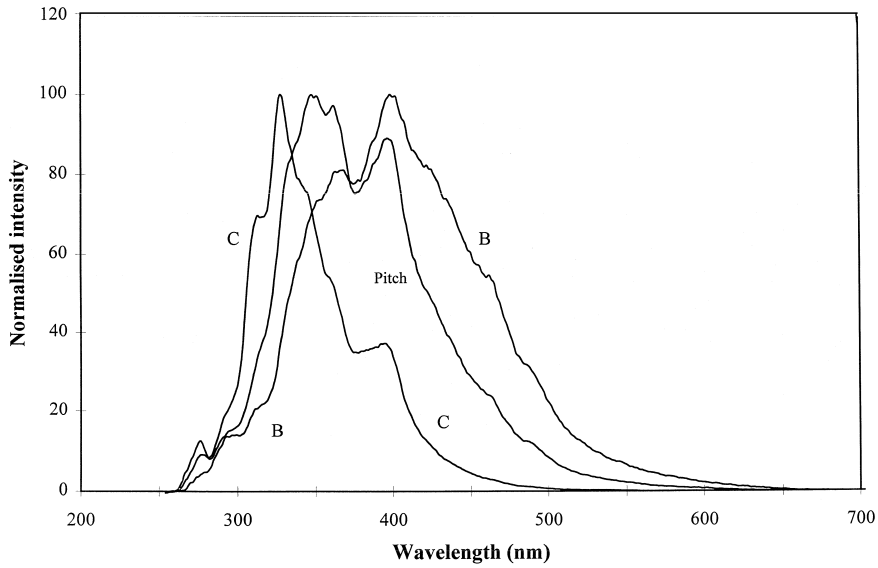

Fig. 3. Synchronous UV-fluorescence spectra of the Stockholm tar and fractions B and C mobile in pyridine and acetonitrile respectively.

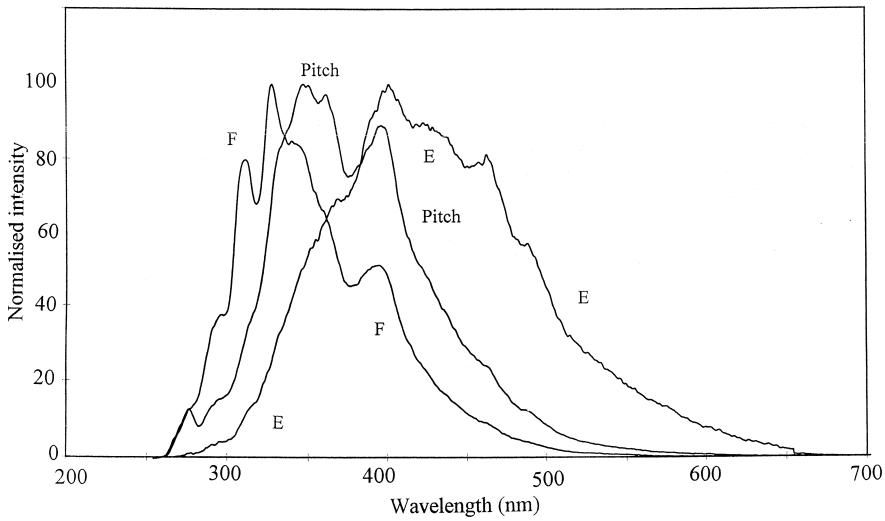

Fig. 4. Synchronous UV-fluorescence spectra of the Stockholm tar and fractions E and F mobile in THF and toluene respectively.

tum yields for the immobile material were very low. This change of fluorescence quantum yield is not readily discernible from Figs. 3 and 4 because the curves have been height-normalised in order to compare the position of the peaks. Larger aromatic ring systems have relatively lower quantum yields: lower signal-to-noise ratios are apparent from these traces.

The UV-F spectrum of the Stockholm tar lies between the spectra of fractions B and C (in pyridine and acetonitrile solvents) and E and F (in THF and toluene solvents), showing that planar chromatography is a powerful method of isolating high molecular mass materials in the less mobile fractions. As the immobile fractions are not the most abundant fractions in the wood tar, relatively higher intensities of the more mobile material tend to mask the relatively weak fluorescence from larger molecules containing more of the larger fused ring systems in spectra of the original (Stockholm tar) sample. This tends to underline the utility of the planar chromatography separation in revealing the immobile materials both rapidly and cheaply.

The UV-F profiles of the most mobile fractions C (Fig. 3) and F (Fig. 4) are similar with maximum intensities near 320 nm. However, the profiles of the fractions mobile in only the first solvent, fractions B and E (Figs. 3 and 4), show different shifts to longer wavelengths; fraction E has the greater proportion of signal between 450 and 650 nm and the lower proportion of signal between 400 and 300 nm. This implies that toluene (fraction F) has mobilised a higher proportion of the fluorescent material than has acetonitrile (fraction C), allowing the fraction immobile in toluene but mobile in THF, with weak fluorescence, to be observed in the absence of material with relatively strong fluorescence around 400 nm. We consider that this shows that the system THF and toluene is the better one to fractionate this biomass tar pitch; the better performance of toluene compared with acetonitrile implies that the mobile material is largely non-polar.

3.4. MALDI-MS

Mass spectra using different matrices have been generated for the whole tar and the fractions. Several ratios of matrix to sample were tried until mass spectra that extended to high masses were generated. Fig. 5a and 5b show the mass spectra of the Stockholm tar with sinapinic acid and with α-cyano acid, respectively. Main peaks with intensity around 1400 u and 2000 u respectively were obtained, with a continuum of ions over the mass range shown; the spectra did not appear to have reached the baseline at 20 000 u (mass limit shown); this corresponds to the exclusion limit of the SEC column.

Mass spectra of the fractions from thin layer

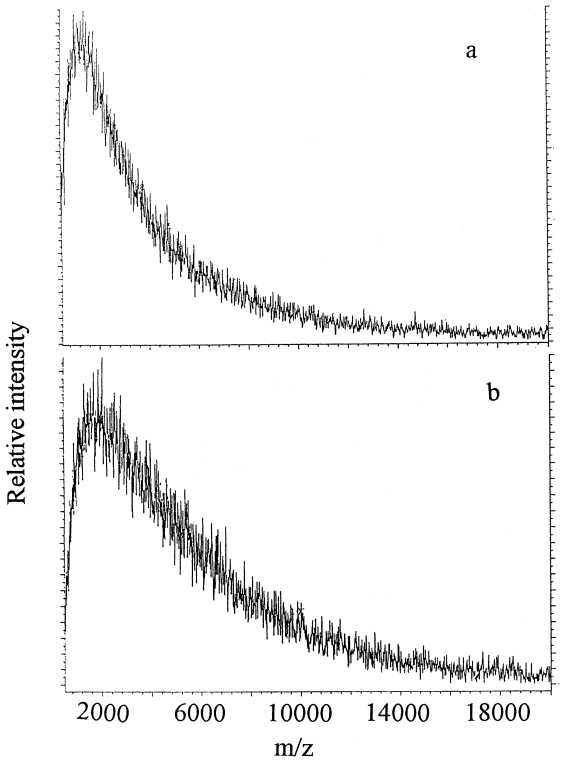

Fig. 5. MALDI-mass spectra of the Stockholm tar a) in sinapinic acid matrix and b) in a-cyano matrix.

chromatography with THF and toluene were generated with 2,5-DHB and in some cases with AgTFA as well (spectra not shown). For fractions B,C and E,F the mass distribution decreased as mobility increased, as indicated by SEC. An ion intensity maximum around 7000 u was observed for fraction E. For fractions A and D, the maximum intensity was observed at about 1000 u; the mass range indicated by SEC for fractions A and D was large and the signal due to higher-MM material is known to be at least partially suppressed [3] in the presence of lower-MM material, particularly when the polydispersity of the sample is much greater than 1.2. The polydispersities of the sample and fractions of the Stockholm tar are not known but are likely to be much greater than 1.2.

Alternative target preparation methods with the use of different matrices may eventually allow generation of satisfactory spectra from the intractable fractions of biomass tars. This work highlights the problems of both selecting a matrix and of preparing a mixture of sample and matrix with the correct relative concentrations appropriate to the molecular mass range of the fraction. Matrices of a different chemical type may be required for biomass tars rather than those used here and found to be successful for coal derived liquids, a reflection of the completely different chemical types and structures of these black materials. Some of the problems associated with generating MALDI spectra have been discussed elsewhere [21,23–26]

4. Conclusions

The separation of Stockholm tar by planar chromatography using two different solvent systems has indicated that THF-toluene gives the better separation and implies that the small molecules of the tar are largely non-polar. Size exclusion chromatography, UV-fluorescence spectroscopy and MALDI-mass spectrometry of the fractions indicate that the planar chromatographic separation is by molecular size and not just by polarity. The UV-fluorescence profiles of the fractions shift with changing mobility in planar chromatography in a manner similar to fractions from coal-derived materials rather than to fractions from petroleum residues. Molecular masses up to 20 000 u are indicated by MALDI-MS. The application of planar chromatography as a fractionation method for the isolation of the large molecules leads to information about the large molecular mass components which cannot be obtained by other means.

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