AVERAGE MASSES OF PITCH FRACTIONS OF NARROW POLYDISPERSITY FOR THE CALIBRATION OF SEC BY ASSESSMENT OF THEIR MALDI-TOF MASS SPECTRA.

C.A. Islas, I. Suelves, A.A. Herod, R. Kandiyoti

Department of Chemical Engineering and Chemical Technology
Imperial College, University of London, London SW7 2BY, UK

Introduction

This work attempts to establish that calibration of SEC with polystyrene standards provides a reasonable estimate of molecular masses (MMs) of coal liquids. Fractionation is necessary to reduce the polydispersity of the sample; too wide a polydispersity leads to the underestimation of the abundance of the high mass component [1]. Although polar components eluted early in a comparable SEC system [2], this was not the case for the present system [3,4] where a variety of standard molecules with different structural features eluted within about 1 min of the polystyrene calibration line. An alternative approach based on the calculation of signal from MALDI mass spectra of polymers of wide polydispersity [5] has been described in which the signal was considered to be real up to the point where the calculated mass averages failed to increase with increasing mass range of the spectrum.

In this work, narrow time-elution fractions (10 sec) of a coal tar pitch have been collected from a preparative size exclusion column using 1-methyl-2-pyrrolidinone (NMP) as eluent. MALDI mass spectra of these fractions using sinapinic acid as matrix have been evaluated by calculation of number (Mn) and weight (Mw) average molecular masses by three different methods [6], based on i) the standard deviation of noise at high mass, ii) the change of slope of baseline as signal exceeded noise at high mass and iii) the method used by Montaudo [5], for comparison with the peak intensity value of the spectrum (Mp). Analytical SEC, calibrated with polystyrene standards, has been used to determine the polystyrene MM equivalent to the elution time of each fraction.

The results show that the Mn and Mw values calculated by two of the three methods, those based on standard deviation and slope of the baseline, lie close to the Mp values and are very similar to the polystyrene standard masses, up to m/z 3000. The third method of calculation [6] greatly overestimated the Mn and Mw values. The quantitative divergence between MALDI and SEC above m/z 3000 will be discussed.

Experimental

Preparative size exclusion chromatography: A Perkin-Elmer LC 250 isocratic pump was used to maintain an NMP flow rate of 2 ml min^{-1}. The preparative SEC column (600 mm; 25 mm ID) used to fractionate the sample was maintained at 85 °C and connected to a Perkin-Elmer LC 290 UV-absorbance detector at 450 nm. A single run of 100 mins gave enough sample. Narrow fractions corresponding to about ten second elution periods were obtained with three minutes intervals between the fractions. This method was used to minimise overlapping between different fractions and to restrict the total number of sample fractions collected.

Analytical size exclusion chromatography: A Polymer Laboratories Mixed-D (5 μm particle size, 300 mm long, 7.5 mm id.) column was used with an NMP flow rate of 0.5 ml min^{-1}. The column has been calibrated using polystyrene standards of narrow polydispersity (Polymer Laboratories) with the detector set at 264 nm. The calibration showed a linear relation between elution volume and log_{10}[MM] of the polystyrene between the permeation (low mass) limit at 23 min and the exclusion limit (mass of approx 200,000 amu) at 11 min. At higher masses, the relation between log_{10}[MM] and elution time showed a different relationship, with the polystyrene standard of MM 1.85 million eluting at about 9 min. Up to a MM of about ~1100 amu, the low mass calibration by polystyrene standards holds within about ±1 min for a wide series of polycyclic aromatic hydrocarbons, nitrogen and oxygen containing molecules [3,4]. None of the small molecules used as standards - or components of coal liquids, biomass tars or petroleum residues, have been observed to elute after 23 min.

MALDI mass spectrometry: A Fisons VG TOFSPEC mass spectrometer (VG Organic Manchester, UK) fitted with a UV-laser (337 nm) and a VAX 4000-base data system with OPUS software has been used [cf Refs. 1,4 for details]. The spectrometer was used in the linear mode; the use of the reflectron was avoided to increase instrument sensitivity to high-MM. An accelerating voltage of 28 kV was used at maximum laser power. Prior to deposition on the target, sinapinic acid was premixed in NMP in a 4:1 matrix to sample solution volume ratio. The target was then dried in a vacuum oven at 150 °C. The peak mass of each spectrum, Mp, was recorded for plotting against the elution time from SEC of the peak intensity of the fraction. The mass spectra were also evaluated by calculation of the standard deviation of the signal at the high mass limit of the spectrum [6]. The 'safe' upper limit of MALDI-signal was taken to be the signal level which exceeded five times the standard deviation of 'noise' level at high mass. The number and weight average MMs (Mn and Mw, respectively) were calculated up to the high-mass limit defined in this way.

UV-fluorescence The Perkin-Elmer LS50 luminescence spectrometer was set to scan at 240 nm min^{-1} with a slit width of 5 nm; synchronous spectra were acquired at a constant wavelength difference of 20 nm. A quartz cell with 1 cm path length was used. Synchronous spectra are shown in peak-normalised mode. Solutions were generally diluted with NMP to avoid self-absorption effects: dilution was increased until the fluorescence signal intensity began to decrease.

Results and Discussion

SEC and UV-fluorescence profiles of the narrow time fractions: Fractions of 10-sec interval effluent from the preparative SEC column were characterised by analytical-SEC (Figure 1). The fractions gave a set of successive narrow peaks in the retained region (15-22 min) and broader peaks in the excluded region (8-12 mins). The proportion of 'excluded' peak diminished with increasing elution time in the preparative column (i.e. with diminishing molecular mass). Fractions 1 to 8 gave only minor proportions of material in the retained region of the analytical column and were not analysed by MALDI . The retained peaks of fractions 9 to 18 however, showed a steady shift to longer elution times with increasing fraction number. Since the excluded material is indicated by the polystyrene calibration to be beyond the range of the MALDI mass spectrometer used, the mass spectrometry results are assumed to provide indications of MM's of the retained peaks. Synchronous UV-fluorescence spectra in Figure 2 show that the fluorescence shifted to shorter wavelengths with increasing elution times, indicative of progressively smaller polynuclear aromatic ring systems and decreasing MMs.

MALDI mass spectra: Spectra from the whole set of fractions analysed were relatively sharp indicating a relatively narrow polydispersity for the fraction, with the peak maximum shifting to smaller-MM with increasing retention time.

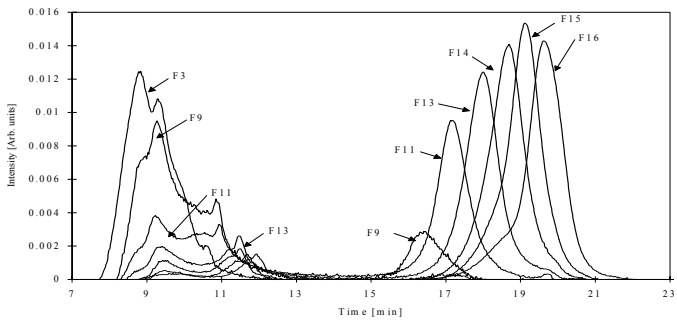

Figure 1 SEC chromatograms of narrow preparative SEC fractions [10 sec] of pitch

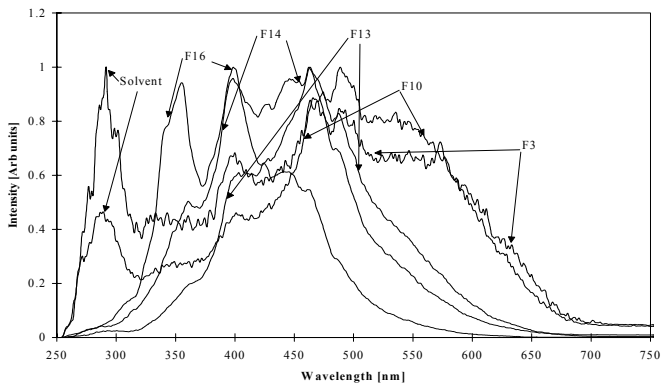

Figure 2. UV-f spectra of narrow preparative SEC fractions [10 sec] of pitch

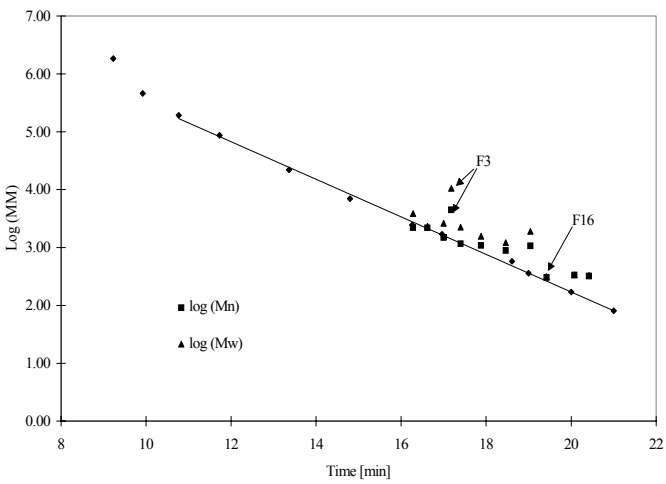

Figure 3 a) Comparison between polystyrene calibration line and Mn and Mw for pitch fractions calculated from MALDI mass spectra

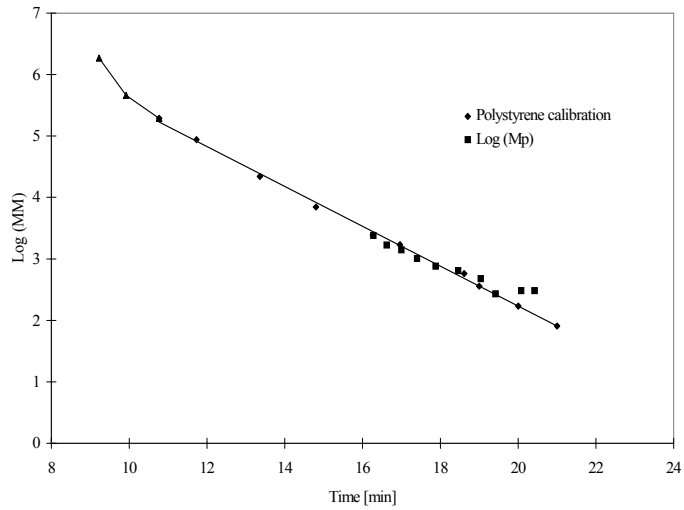

Figure 3 b) Comparison between polystyrene calibration line and Mp from MALDI mass spectra for pitch fractions

Figure 3 shows plots of the times of the peak maximum of the fraction, against the number and weight averages determined from MALDI-ms by each method and against the polystyrene calibration mass. Figure 3a compares the polystyrene calibration line with the Mp values from the MALDI mass spectra. Figure 3b compares the polystyrene calibration with Mn an Mw values calculated from the mass spectra. The Mp values appear to be a better match to the polystyrene equivalent mass values as defined by the elution times of the pitch fractions, although the calculated values gave only a slightly worse match. The polystyrene calibration is seen to give a reliable estimation of the masses of the fractions within the limited range of mass up to m/z 3-4000.

Conclusions

Preparative SEC of a pitch sample was used to collect fractions of narrow polydispersity. Mass spectra of the fractions have been obtained by MALDI-ms. Peak mass values (Mp), number and weight average masses (Mn, Mw) have been calculated from these spectra. These values have been compared with MMs of the fractions, calculated from their elution times in analytical SEC, using a calibration by polystyrene standards. The comparison shows quantitative agreement between molecular masses of pitch fractions from the polystyrene calibration and molecular masses found by MALDI-ms, up to about 3000 u.

References

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