Application of approach to equilibrium analytical ultracentrifugation (Archibald method) to the determination of molecular weights of humic substances.

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Humic substances (fulvic and humic acids) are the decomposition products of plant material and constitute a major part of the earth's organic carbon [1]. Their presence in aquatic ecosystems is important because of their binding capacity for metals, organic pollutants such as pesticides and herbicides [2]. The characterisation of humic substances in terms of molecular weight and molecular weight distribution is a difficult problem due both to the width of the molecular weight distributions in humic samples (several hundreds up to millions) [3], and to the tendency of humic substances to associate in aqueous media. In a recent study [4] the technique of analytical (UV scanning) ultracentrifugation was applied to the characterisation of a range of humic samples from peat and surface water. This technique successfully exploits the large extinction coefficients of humic substances in the UV spectral region allowing measurements to be made at very low concentrations (~ 45 mg l⁻¹), comparable to those found in natural waters. The method has limitations for samples of high molecular weight as not all the sample is contained in the measurable concentration gradient (OD 0–1), and hence the information relates only to the lower end of the molecular weight distribution. In an attempt to overcome this problem we have investigated the application of the approach to equilibrium (Archibald) method to humic samples. This method depends on the fact that the conditions for sedimentation equilibrium are realized at all times at the ends of a fluid column. Hence if a measurement of the concentration (c) and concentration gradient (dc/dr) can be made at those positions the average molecular weight (Mₐ) can be calculated after a short period of centrifugation [5]. Thus from measurements made at the meniscus (m), Mₐ can be calculated from the equation:

\[ M_a = \frac{RT}{(1 - \phi \rho) \eta_m} \frac{1}{n_m} \left( \frac{dc}{dr} \right)_m \]

Where R, T, ρ, φ, and n_m are the gas constant, absolute temperature, partial specific volume, solvent density, angular velocity and distance of the meniscus from the axis of rotation respectively. The advantage of this technique for humic materials is that measurements are made at short time periods before any high molecular weight material is lost from the measurable concentration gradient at the cell base where OD > 1, and hence Mₐ should be close to that for the whole sample.

To test the method measurements have been made on low molecular weight humic and fulvic acids and a high molecular weight humic acid, and the results compared with sedimentation equilibrium measurements. The low molecular weight humic and fulvic acids were extracted from water in a drainage pond at the Broubster natural analogue site, Thurso, Scotland. About 2,000 litres of water were passed through a radial–flow column packed with the weak–base ion exchange resin DEAE–cellulose. The sorbed humic material was eluted with 0.5M NaOH, isolated, and purified by precipitation with HCl. The fulvic acid was purified by sorption onto SM–2 Biobeads® followed by elution with MeCN which was subsequently removed by rotary evaporation. The high molecular weight sample (kindly supplied by Dr. A. Majid, National Research Council of Canada) was extracted from the Alberta oil sands. The samples were analysed using a Beckman L8–70 ultracentrifuge fitted with a UV scanner, run at 35,000 rpm (Archibald method) and 25,000 rpm (equilibrium method) in the case of the low molecular weight samples and 10,000 rpm (both methods) in the case of the high molecular weight sample.

Fig. 1. Comparison of the approach to equilibrium (Archibald) method with sedimentation equilibrium for low molecular weight fulvic (--) and humic (•) acids and a higher molecular weight humic acid (△). Archibald results shown as open symbols and equilibrium results as closed symbols.

Fig. 1 shows the values of Mₐ for several time intervals which after extrapolation to zero time are compared with the values obtained at sedimentation equilibrium. For the fulvic acid where 93% of the sample was in the measurable concentration gradient at sedimentation equilibrium, the agreement between the two methods is very satisfactory, confirming the reliability of the Archibald technique (Mₐ = 1610 ± 55). The lower Mₐ humic sample gave an Mₐ = 4415 ± 250 on extrapolation to zero time, whereas sedimentation equilibrium gave a lower value (Mₐ = 3811 ± 38) because only 6.3% of the material could be measured. For the high molecular weight humic sample the Archibald method gave an Mₐ ~ 70,000 which was very much higher than the sedimentation equilibrium value of ~ 10,000, in this case only 48% of the sample contributed to the equilibrium result.

These results demonstrate that the Archibald method is the only ultracentrifugation method currently available which will give reliable weight average molecular weights for whole samples of the larger humic materials.


