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## Note

### Application of electrochemical and ultraviolet detectors in the gel filtration of humic acids

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Humic substances, and more specifically fulvic and humic acids, have been subject of intense research because of their important significance in agricultural and environmental processes<sup>1-4</sup>. These materials interact not only with naturally occurring chemicals but also with chemicals introduced into the environment. The humic substances consist of two categories of acidic compounds: the fulvic acids of lower molecular weight (200-1000)<sup>5</sup>, lower equivalent weight and correspondently high charge density; and the humic acids with molecular weight up to 200,000 of correspondingly larger size, higher equivalent weight and a low charge density<sup>6</sup>. The model humic compound is generally taken as constituted of a central polyhetero condensate of organic moieties<sup>7</sup> with peripheral polar functional groups including carboxyl, phenolic hydroxyl, enolic hydroxyl, alcoholic hydroxyl, quinone, hydroxyquinone, other carbonyl, ester, lactone, ether and amine groups. Of these the carboxyl and acidic hydroxyl groups are the most relevant in the binding of the metal ions<sup>8</sup>. Because of these interactions the chemical properties of metal ions are markedly modified and thus natural processes may be affected. The macromolecular structure of humic acids has been studied by a variety of techniques, such as X-ray scattering<sup>9</sup>, gel chromatography and viscosity, dialysis and ultrafiltration<sup>10,11</sup> and chemical analysis<sup>12</sup>. In studying humic materials it should be kept in mind that these substances are exceedingly complex mixtures, and despite a great deal of work, the exact structure of humic substances is not yet clear. The present study used electrochemical detection combined with molecular size exclusion to investigate the question of humic acid structure. We have compared the behaviour of two commercial humic samples of a natural soil extract and of a "synthetic" humic acid prepared from hydroquinone. We also aimed to evaluate the relative responses of the ultraviolet and the electrochemical detector; no attempt was made to optimize the chromatographic separation conditions.

#### EXPERIMENTAL

##### *Apparatus*

Two detectors were used in the study. An electrochemical detector, Metrohm

Model 656 equipped with a three-electrode detection cell, Model EA 1096/2. A Metrohm VA 641 potentiostat was used and the detector output was displayed on a Houston Omniscrite recorder. The working electrode was a carbon paste electrode; it was preferred to the glassy carbon electrode for its better reproducibility and low residual current. The UV detector was a Hewlett-Packard Model 1032 at 254 nm.

### Reagents

Humic acid samples were purchased from Aldrich and Fluka. Hydroquinone (Carlo Erba) was used to prepare synthetic humic acid. The model compounds for the calibration of the column were analytical reagents used without further purification.

### Column and sample preparation

Glass columns ( $25 \times 16$  mm I.D.) were packed with Ultragel LKB (exclusion limits 1000–15,000) according to instructions of the manufacturer. As eluent a solution of 0.05 M sodium pyrophosphate was used with a gravity flow-rate of *ca.* 0.5 ml/min. Compounds of known molecular weight and chemically similar to groups believed to be present in the humic structures were used to calibrate the Ultragel columns. The test compounds for the molecular weight distribution analysis, blue dextran (MW  $2 \cdot 10^6$ ), thymol blue (MW 466), 4,4'-dihydroxybenzophenone (MW 214) and phenol (MW 94) were dissolved in the eluent at a concentration of 1 mg/ml.

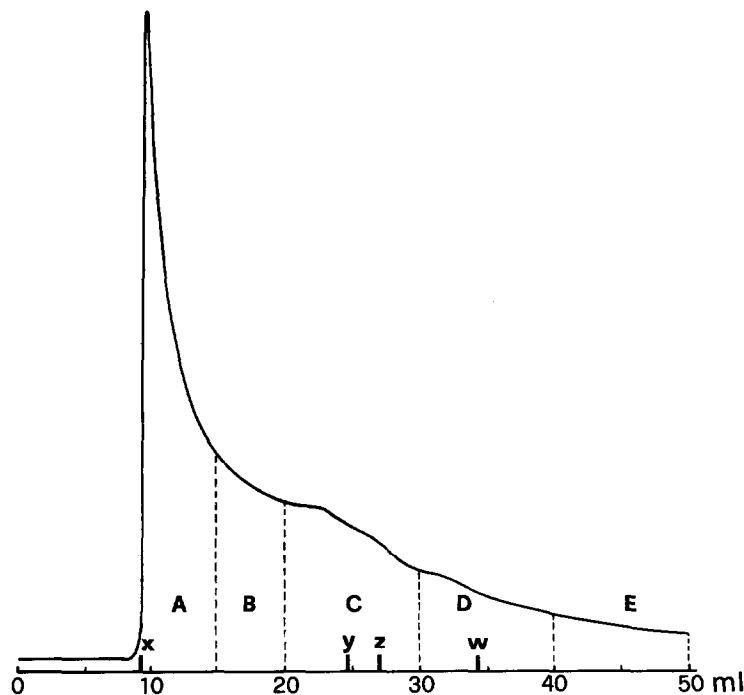


Fig. 1. Elution curve of Aldrich humic acid. Sample volume, 0.05 ml of 0.05% solution. UV detector. Points x, y, z and w indicate the retention volumes of blue dextran, thymol blue, 4,4'-dihydroxybenzophenone and phenol.

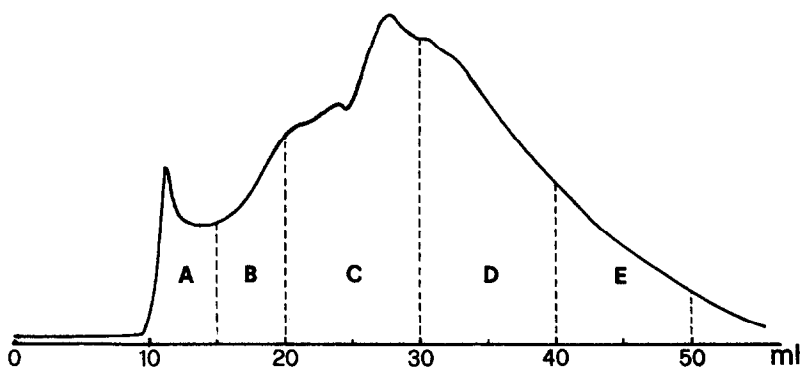


Fig. 2. Elution curve of Aldrich humic acid. Sample volumes, 0.5 ml of 0.5% solution. Electrochemical detection. Electrode potential, +1.0 V vs. Ag/AgCl; sensitivity, 0.5  $\mu$ A full scale.

Synthetic humic acid was prepared according to Curtis *et al.*<sup>13</sup>. The sample of natural humic acid was obtained from a typical peaty area following the method of Kononova<sup>1</sup>.

#### RESULTS AND DISCUSSION

Figs. 1, 3, 5 and 7 show the UV responses of the Aldrich, Fluka, peaty and synthetic samples under the reported experimental conditions. Figs. 2, 4, 6 and 8 show the corresponding electrochemical curves. To make the differences between the two detectors clearer, we have divided the chromatograms into five regions relating to decreasing molecular size (A, B, C, D, E). Table I summarizes the data relating to these distribution regions. The comparison between the two sets of curves reveals the following features.

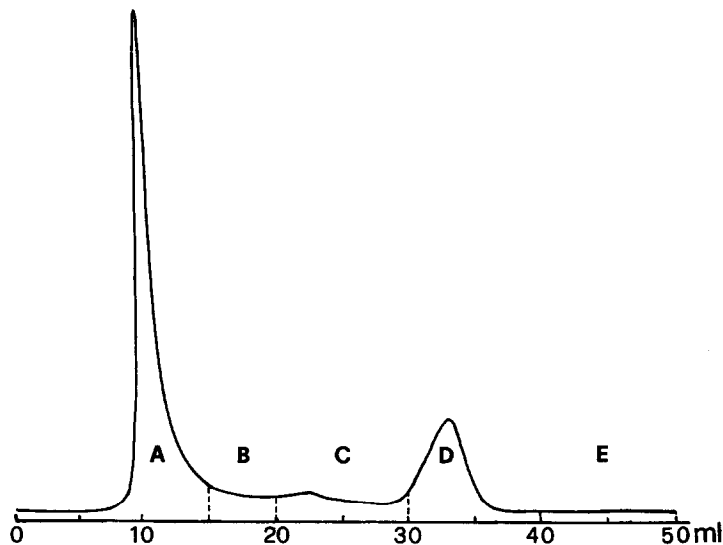


Fig. 3. Elution curve of Fluka humic acid. Sample volumes, 0.05 ml of 0.05% solution. UV detector.

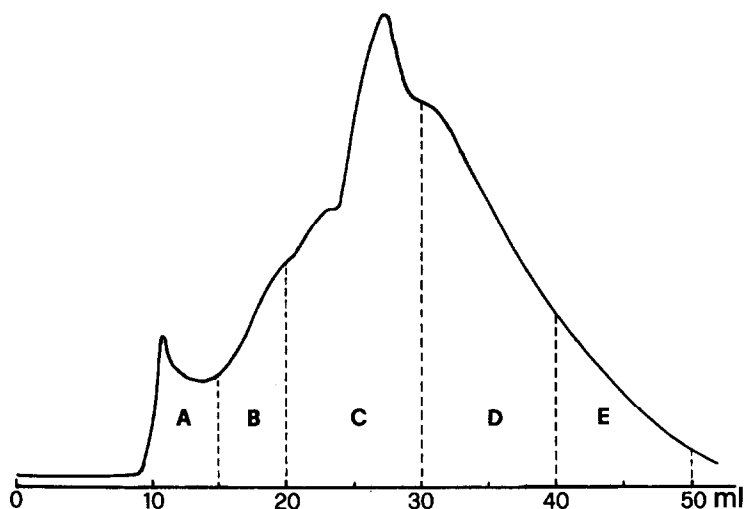


Fig. 4. Elution curve of Fluka humic acid. Sample volume, 0.5 ml of 0.5% solution. Electrochemical detector, see Fig. 2.

(1) The region A, which contains the highest molecular weight humic structures, gives a higher response to the UV detector than the electrochemical detector. With the UV detector (apart from the synthetic sample) this fraction is the most abundant.

(2) In the lower molecular size regions, particularly C, D and E, the electrochemical response is much higher than the UV response.

(3) The chromatogram produced by the synthetic sample has a special pattern. The reaction products of hydroquinone in basic aqueous solution are believed to

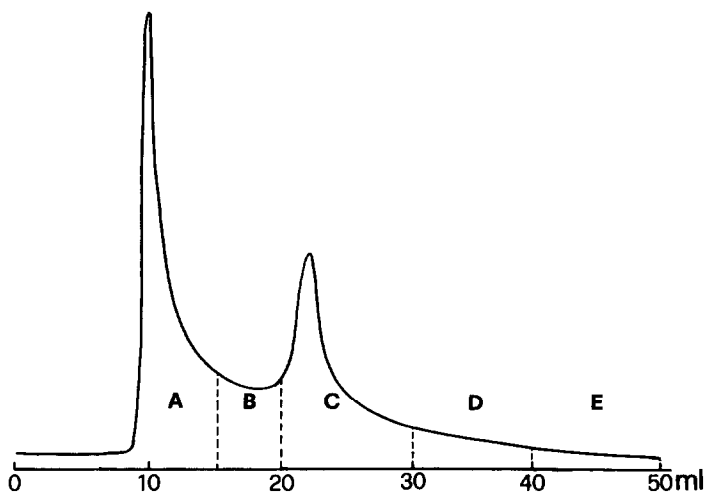


Fig. 5. Elution curve of peaty soil extract. UV detector.

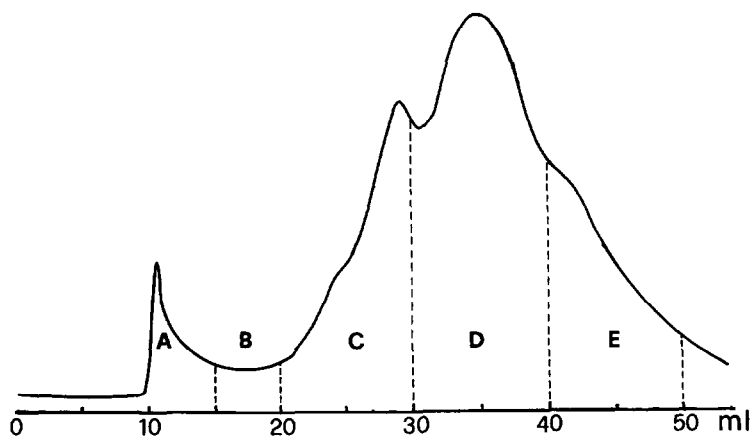


Fig. 6. Elution curve of peaty soil extract. Electrochemical detector, see Fig. 2.

TABLE I

PERCENTAGE OF EXCLUDED FRACTION FOR THE VARIOUS REGIONS OF THE ELUTION CURVES

<i>Sample</i>	<i>Detector</i>	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>
Aldrich	UV	39.0	17.5	25.6	11.4	6.5
	EC*	7.9	10.4	36.0	32.0	13.7
Fluka	UV	66.2	6.0	8.7	19.0	0.0
	EC	6.0	8.5	41.1	33.2	11.2
Peaty soil	UV	41.4	13.1	37.9	6.5	1.0
	EC	5.2	2.6	23.4	48.6	20.2
Synthetic	UV	1.7	2.0	51.1	36.8	8.4
	EC	0.0	0.8	29.0	67.0	3.2

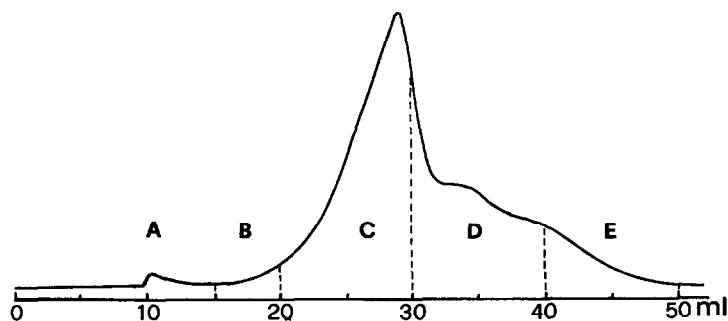


Fig. 7. Elution curve of synthetic humic acid. UV detector.

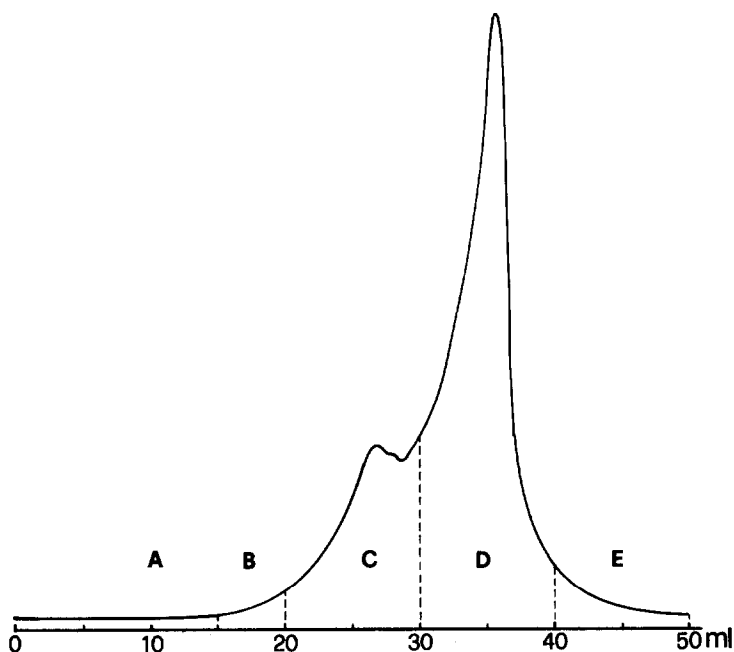


Fig. 8. Elution curve of synthetic humic acid. Electrochemical detector, see Fig. 2.

consist of fairly short phenolic polymers of relatively low mean molecular weight. In this case the differences between the two responses are not striking. The electrochemical detector is notoriously selective for oxidisable organic groups. In particular, the phenolic and aromatic amino groups give a high responses with such devices<sup>14,15</sup>. So it is reasonable to suppose that the lower molecular weight fractions contain a relatively large number of oxidisable groups (e.g. phenolic and aromatic groups) relative to the fraction containing the largest structures. This conclusion agrees with recent literature data<sup>12</sup> obtained by different analytical techniques.

The technique shows considerable promise for the quantitative evaluation of the total oxidisable compounds if one takes in account the response of a standard substance (e.g. phenol). Work in this direction is in progress.

#### ACKNOWLEDGEMENTS

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