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Adsorption Effects in Gel Filtration of Humic Acid

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The remarkable success of the gel filtration method in the study of a number of complex systems has caused its application also to the investigation of the ill-defined organic matter in soil which is usually called humus or humic acids.¹⁻¹⁸ This presumably polyaromatic and certainly polyelectrolytic matter offers special difficulties in the evaluation of the gel filtration results. These difficulties are not always fully realized by those who use the method.

All aromatic compounds are to some extent adsorbed by Sephadex gels and the polyaromatic nature of the humic acids

leads to serious adsorption effects. Posner¹² has correctly pointed out that adsorption takes place in salt medium but his conclusions about a suitable elution procedure have not been corroborated by the present study.

Experimental. A great number of humic acid samples of different origin and obtained by different extraction methods have been studied. The same adsorption effects were found in all cases and only two typical examples will be presented here. The sample used was a neutral solution of the most salt-sensitive part of the humic acid obtained from a *chernozem* soil. Sephadex G15 and G100 from Pharmacia have been used for the gel filtrations. The columns had the dimensions 38 × 2.5 cm. The elution velocity was controlled by a peristaltic pump to 24 ml/h. The columns were always equilibrated with the first elution solution before the start of the elution.

The UV absorption at 253.6 nm was registered on an LKB Uvicord instrument. 3 ml of the samples were added to the column.

Results and discussion. We will first treat the case without any other separation than that due to adsorption. This is exemplified by the gel filtrations on G15 with this particular sample. By elution with 0.05 M NaCl (Fig. 1a) only one fraction is obtained even after 24 h of elution or more. A change of eluent to distilled water leads, however, to the development after some hours of a clearly visible fraction which is also registered at the Uvicord diagram. The appearance of this fraction coincides with the disappearance of salt in the eluted solution as found by conductance measurements.

It thus seems as if the humic acid molecules in the salt solution are strongly adsorbed on the available adsorption sites of the gel and not until all adsorption sites are occupied the rest of the sample will pass through the column completely excluded. At the subsequent elution with distilled water the humic acid molecules are released from the adsorption sites. They are then accumulated in the front of the solution with low ionic strength and there gradually form a rather sharp fraction.

This interpretation is corroborated by the following experiment. The excluded fraction was concentrated to the original volume and rerun through the column in exactly the same way as earlier (Fig. 1b). The same amount was adsorbed and only a small fraction was completely excluded.

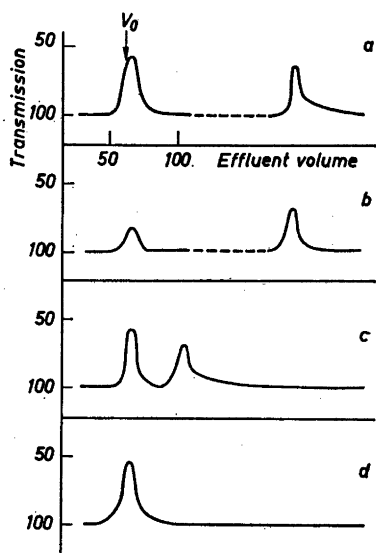


Fig. 1. Gel filtration of a humic acid on Sephadex G15 under different conditions.

- Elution of a humic acid sample first with 0.05 M NaCl and later with distilled water.
- Elution of the first fraction in *a*, first with 0.05 M NaCl and later with distilled water.
- Elution of the sample with distilled water after addition of 1 M NaCl.
- Elution of the sample with distilled water after dialysis.

The procedure suggested by Posner,¹² to give the sample a high ionic strength and then to elute with distilled water leads to a serious misinterpretation of the gel filtration results in this case. The separation obtained (Fig. 1c) is an artefact obtained due to the adsorption of humic acid molecules in salt medium. The second fraction is thus always preceded by a striking decrease in conductance. If the ionic strength is reduced the separation will gradually disappear and with a dialyzed sample only one, completely excluded fraction is obtained (Fig. 1d). Similar results have earlier been reported by Dell'Agnola and Maggioni.³

The experiments with the G100 gel represent the case with real separation, which, however, is complicated by adsorption effects. By elution with 0.05 M NaCl two fractions are obtained (Fig. 2a) but also

a third fraction after change to distilled water as eluent. Both the completely excluded and the retarded fractions are adsorbed in reruns (Figs. 2b and c). Elution according to Posner¹² leads to a sharp fractionation (Fig. 2d), but the second fraction which appears when the salt has been eluted must also contain the adsorbed fraction of the high-molecular fraction (*cf.* Figs. 1a, b, and c; *cf.* also the results by Dell'Agnola and Maggioni³). A dialyzed sample eluted with distilled water, finally, is only incompletely separated in two fractions (Fig. 2e).

The increased exclusion of the low-molecular fraction with decreasing ionic strength and the adsorption at higher ionic strengths have been extensively studied by Eaker and Porath,¹⁷ and will not be further

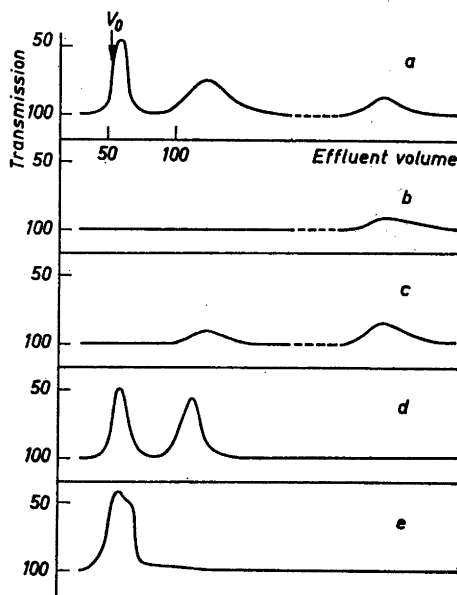


Fig. 2. Gel filtration of a humic acid on Sephadex G100 under different conditions.

- Elution of a humic acid sample first with 0.05 M NaCl and later with distilled water.
- Elution of the first fraction in *a*, first with 0.05 M NaCl and later with distilled water.
- Elution of the second fraction in *a*, first with 0.05 M NaCl and later with distilled water.
- Elution of the sample with distilled water after addition of 1 M NaCl.
- Elution of the sample with distilled water after dialysis.

treated here. The main purpose of this study has been to point out the existence of a very strong adsorption of both high- and low-molecular molecules on the outer parts of the Sephadex gels.

The two possible procedures which can be employed in gel filtration of humic acids seem to be, either to elute with a salt eluent (as done in Refs. 4, 6, 8, 9, 11, 13, and 14) and discard the strongly adsorbed part (containing both high- and low-molecular components) or to elute dialyzed samples with distilled water³ (obtaining a less efficient separation). The main rule must be to avoid ionic strength gradients during the separation. This is also emphasized by Eaker and Porath.¹⁷

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Studies on Sphingosines

15. Degradation of Phytosphingosine to Hydroxy Fatty Acid and Ethanolamine by the Yeast *Hansenula ciferrii*

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The first experimental evidence for an enzymatic degradation of sphingolipid long chain bases was presented by Korey and Stein,¹ who studied an enzyme system of rat brain, capable of degrading gangliosides stepwise, including the long chain bases. Very recently indications for fatty acids as catabolites of long chain bases were given.²⁻⁴ Phytosphingosine (1,3,4-trihydroxy-2-amino-octadecane) was degraded to hydroxy fatty acid.⁴ Formally, the remaining part is 2-aminoethanol. Alternatively, a two-step one carbon degradation, possibly preceded by a deamination, should be considered. The present communication gives evidence for ethanolamine as a catabolite of phytosphingosine in the yeast.

Hansenula ciferrii (F-60-10)⁵ was grown for 4 days at 25°C in yeast maintenance broth with ¹⁴C-phytosphingosine,⁴ prepared from the yeast given 3-¹⁴C-serine. The incubation mixture was extracted with chloroform-methanol 2:1, v/v, and the insoluble cell residues were filtered off. The extract was partitioned against water (chloroform-methanol-water 8:4:3, v/v/v). The upper (I) and lower phases were separately taken to dryness. The upper phase (I) was hydrolyzed for 13 h in 1 M HCl in water and evaporated. The lower, lipid phase was hydrolyzed for 6 h in 2 M HCl in water and partitioned as described above. The upper phase (II) was evaporated. Fatty acids and long chain bases in the lower phase were separated and purified using silicic acid column and thin layer chromatography (Table 1).⁴

The two upper phases (I and II) were separately subjected to dinitrophenyl (DNP) synthesis⁶ and the products extracted with diethyl ether before (a) and after (b) acidification with hydrochloric acid. In II all radio-