Radio-chromatography in Migration Studies

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Data Sheet

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RESUMÉ

Væskekromatografi i kombination med radioaktivitets detektion, dvs. radiokromatografi, er en let tilgængelig eksperimentel teknik til bestemmelse af vigtige fysisk-kemiske egenskaber som permeabilitet, porositet, dispersions-, diffusions- og sorptionskarakteristika for geologisk materiale. Anvendelsen af konventionel radiokromatografi kan med fordel anvendes til studier af miljørelaterede processer.

Principperne for radiokromatografi er diskuteret og efterfølgende illustreret med udvalgte eksempler, der omhandler cæsium migration i kalk, europium sorption til knust salt, iodering af humus syrer og phenol samt enzymatisk inkorporering af phenol i humus syrer.

SUMMARY

Liquid chromatography in combination with radioactivity detection, i.e. radiochromatography appears as a facile experimental technique to determine important physico-chemical properties such as permeabillity, porosity, dispersion-, diffusion-, and sorption charateristics of geological material. The application of conventional radiochromatography appeas advantageous in studies of environmental processes.

The basic principles of radiochromatography are discussed and subsequently illustrated by selected examples, comprising cesium migration in chalk, europium sorption to crushed salt, iodination of humic acids and phenol, and the enzyme mediated incorporation of phenol in humic acids.

1. INTRODUCTION

Radiochromatography, i.e. the combination of liquid chromato-
graphy and radioactivity detection, constitute as an efficient tool
in evaluation of factors of importance to migration behaviour of
pollutants in the environment.

Liquid chromatography is a column technique normally used for
the separation of different organic as well as inorganic compo-
nents in a solution.

A typical experimental set-up for a liquid chromatographic separ-
ation is shown in Figure 1. The pump delivers a solvent (eluent)
flow at a preset, constant flow rate. Samples of known volume are
injected into the column via the injection port and the eluate from
the column is continuously monitored by a detector.
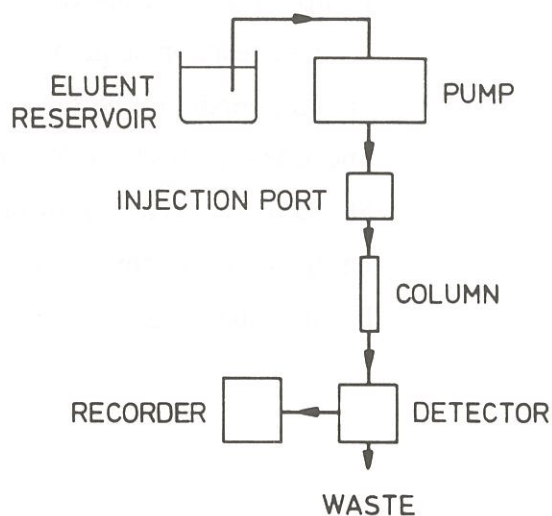
Fig.1: Experimental Liquid Chromatography set-up

For migration studies radiochromatography can be applied in two
different ways, depending of the column material used. Thus, the
system can be used to characterize geological material, such as

chalk (Carlsen et al., 1981, Carlsen and Batsberg, 1982, Carlsen et al., 1985) or fractured granite (Carlsen and Platz, 1985) with respect to permeability, volume porosity and flow dispersion as well as to study the interaction between the geological material and selected solutes, e.g. radionuclides, in order to evaluate the possible migration potential through the media.

For a given column material, e.g. chalk, the separation mechanism will be controlled by the physical and chemical nature of the interaction between the molecules present in the injected sample and the column material. Adsorption-, partition-, substitution-, and ion exchange processes, separately or in combination, can occur in the columns, being responsible for the actual shape of the chromatogram. Figure 2 depicts a typical chromatogram used for the determination of the different physico-chemical characteristics of the columns.

Alternatively, conventional liquid chromatography columns can be applied in studies of e.g. speciation of radionuclides following a given reaction sequence, as illustrated with studies on the enzyme mediated halogenation of humic material (Christiansen and Carlsen, 1991a, 1991b, Carlsen and Lassen, 1992., Lassen et al., 1991a..), a reaction of potential importance in the fate of halogens in the environment, and incorporation of phenol in humic substances (Lassen et al., 1991b).

2. COLUMN CHARACTERIZATION

The shape of the peak corresponding to an unretarded solute characterizes the flow dispersion in the column whereas the elution volume (or time) as well as the shape for a retarded peak characterizes sorption phenomena, related to the solute under investigation, on the column packing material. Symmetrical peak shapes are normally obtained in cases where a single mechanism, with a concentration independent distribution coefficient, can describe. the sorption phenomena. Cases where several mechanisms are operating simultaneously will generally lead to skewed peaks.

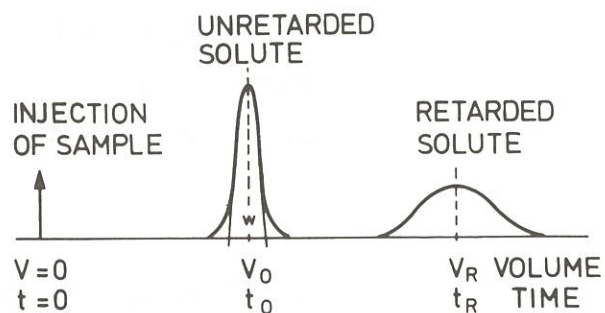*Fig.* 2: Schematic Chromatogram defining elution volume and time.

2.1 Permeability:

The permeability, k for a given column (length L and cross-section area A) can be calculated by determination of the pressure drop over the column, h, for a certain volume flow-rate, v (equation (1)), h being measured in units of meter of water.

$$k = v \cdot L / A \cdot h \text{ (cm/sec)} \tag{1}$$

2.2 Porosity:

The volume porosity, ε, of a column can be determined by injection of a solute, which is not retarded by the column material, according to equation (2)

$$\varepsilon = V_O / A \cdot L \qquad (2)$$

Where V_O is the elution volume for a non-retarded solute, A is the column cross-section area, and L is the length of the column. In the case of chalk columns the porosities were determined by injection of D_2O (30% in H_2O,) onto consolidated chalk column primary equilibrated with H_2O, V_O being measured by a refractive index detector. Alternatively, $^{36}Cl^-$ was injected onto the columns, the elution volumes being detected by a radioactivity monitor. The hereby measured elution volumes were identical to those found by application of D_2O. Additionally, porosities can be calculated based on density determinations.

2.3 Flow dispersion:

The flow dispersion in the column, σ, is the case of Gausian peaks, equal to the standard deviation, determined by the width of the peak (at the base line), w, (Yan et al. 1979) the σ-w relation being given by equation (3)

$$\sigma = w/4 = \omega V_O / 4 \qquad (3)$$

where $\omega = w/V_O$, i.e. the flow dispersion is calculated in units of the pore volume V_O,

2.4 Sorption:

In the column experiments the retention factors, R_f, are calculated as the ratio between the elution volume of the retarded species, V, and that of the non-retarded species, V_O, (equation (4)), corresponding to the ratio between the ground water velocity and that of the retarded species. (Relyea,1982)

$$R_f = V/V_O \tag{4}$$

In batch type experiments distribution coefficients, K_D, are determined for crushed samples by the procedure previously described, (Bo and Carlsen, 1981), the K_D values being transformed into R_f values due to the K_D - R_f relation (equation (5)), adopting a bulk $CaCO_3$ density, ρ, as 2.7 g/cm^3.

$$R_f = 1 + (1-\varepsilon) \cdot \rho \cdot K_D/\varepsilon \tag{5}$$

As an illustrative example the sorption of $^{134}Cs^+$ by Erslev Maastricht Chalk as a function of sodium chloride content in the eluate can be mentioned (Fig. 3) (Carlsen et al., 1985)

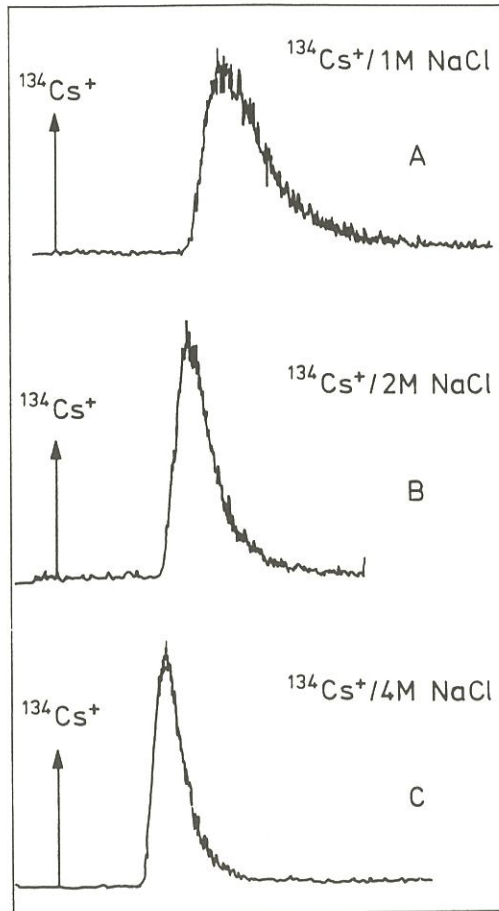*Fig. 3*: Retention of $^{134}Cs^+$ in a 1 cm Erslev Maastricht Chalk column as funktion of the eluent sodium chloride concentration, visualized as $^{134}Cs^+$ concentration (cps) as function of elution volume (V). (Flow-rate: 0.3 mL/min.).

Similar effects are noted for other radionuclides (Carlsen et al., 1981, Carlsen and Batsberg, 1982, Carlsen et al., 1985).

2.5. Columns with very low permeabilities.

In the case of studies on samples with very low permeabilities, e.g. fissured granite some modifications of the system have been introced. Figures 4 and 5 illustrate the experimental set-up and a close-up of the compression module with the fissured column, respectively (Carlsen and Platz, 1985).

In the original approach (vide supra) the pressure drop over the column was determined by the permeability of the column and the flow rate through the column. Thus, the pressure drop could be controlled only by the flow rates available by the pump. The modified system, however, allows adjustment (by the needle valve A, cf. Figs.4 and 5) of the pressure drop over the column (measured by the manometer M_1, cf. Fig. 4) to the desired value. The eluent (delivered by the pump P_1, cf. Fig. 4) passes, at a relatively high flow rate (0.5 - 1.0 ml/min), over the column end surface in a small chamber of a volume of about 0.3 ml (cf. Fig. 5, B).
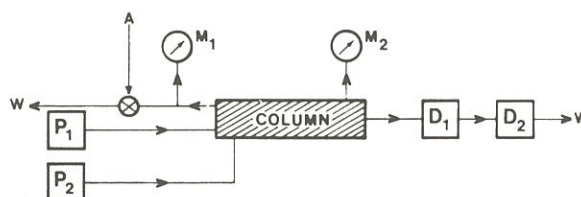
Fig. 4: Experimental set-up for liquid chromatography investigations. P_1: pump to deliver the flow through the column, the pressure drop over the column being controlled by the needle valve A, and measured by the manometer M_1. P_2: pump to deliver hydrostatic pressure on the column, which can be measured by the manometer M_2. D_1 and D_2 denote the radioactivity - and ultraviolet detection systems, respectively, w denotes waste.

The amount of eluent passing through the column (to "out-2", cf. Fig. 5) relative to the amount passing through the valve to waste (denoted by "out-1", cf. Fig. 5) was determined by appropriate

adjustment of the needle valve A. Apart from the possibility of arbitrary choice of pressure drops, the present set-up ensures that the full surface at the inlet side constantly is in contact with the eluent. Furthermore, the dead-volume of the system can be kept rather low (ca. 0.65 ml).

The hydrostatic pressure on the column (delivered by pump P_2) can be adjusted to mimic a certain depth in the actual geologic formation. It is important to note that the hydrostatic pressure must be higher than the pressure drop over the column to prevent channeling along the surface of the column.
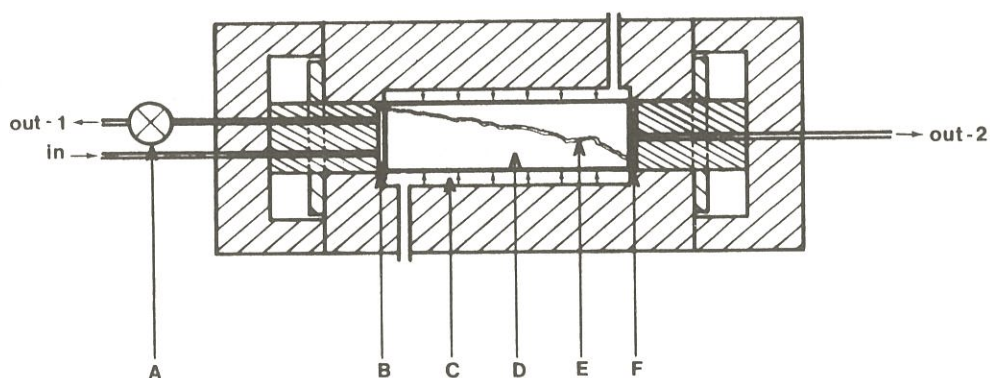
Fig. 5: Compression module with granite column. The column and the inlet/outlet joints are encapsulated in shrinkable polyethylene tubing. A: needle valve to control the pressure drop over the column (P_i), B: 1 mm spacer, C: liquid (e.g. water) to maintain the hydrostatic pressure on the column (P_O), D: column, E: fissure, F: filter paper.

The evaluation of the resulting chromatograms is similar to the above mentioned.

3. HIGHLY CORROSIVE ELUENTS

In cases, where the eluent are highly corrosive towards the chromatography system, as e.g. high concentration brines, the experiments advantageously can be carried out using a classical column technique combined with fraction sampling, followed by separate counting of the single samples. The studies on radionuclide migration through salts columns constitute in this context as an illustrative example (Carlsen and Platz, 1986, Carlsen, 1987).

The experiments were either carried out as single pulse injections, where a small portion of a saturated sodium chloride solution containing the radionuclide/carrier in a known concentration was applied to the top of the column or as break-through-experiments (continuous flow injection) where the radionuclide/carrier (known concentration) continuously was applied to the column in pre filtered saturated sodium chloride. The eluate is collected in ca. 0.5 mL portions and the radionuclide concentration determined by γ-counting. Eventually, the columns were sectioned (approx. in 0.4 g fractions), the salt being weighted and dissolved in pure water, the amount of radionuclide being determined by γ-counting. The applied column system is visualized in fig. 6.
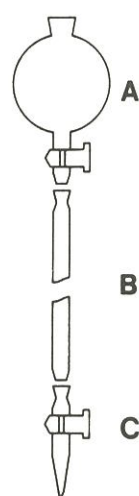
Fig.6: Separated Column system: A: Reservoir Bulb, B: Column Tube, C: Outlet Valve.

The system was used to elucidate whether the sorbed europium and americium are strongly or loosely bounded to the halite. The study was carried out as sorption/desorption experiments. In Figure 7 the elution curve (continuous flow injection) for the europium sorption/desorption experiments is shown: after applying 250 mL of the europium containing saturated sodium chloride solution, 250 mL of pure saturated sodium chloride solution was passed through the column. Obviously europium is sorbed to the halite since the concentration of europium in the eluate reaches only ca. 60%. Apparently, application of the pure brine afforded an instantanous drop in the eluate europium concentration (Figure 7, last part). Consequently, it was expected that a major part of the europium was still to be found on the column matereial, in nice agreement with the experimental result (Figure 8).
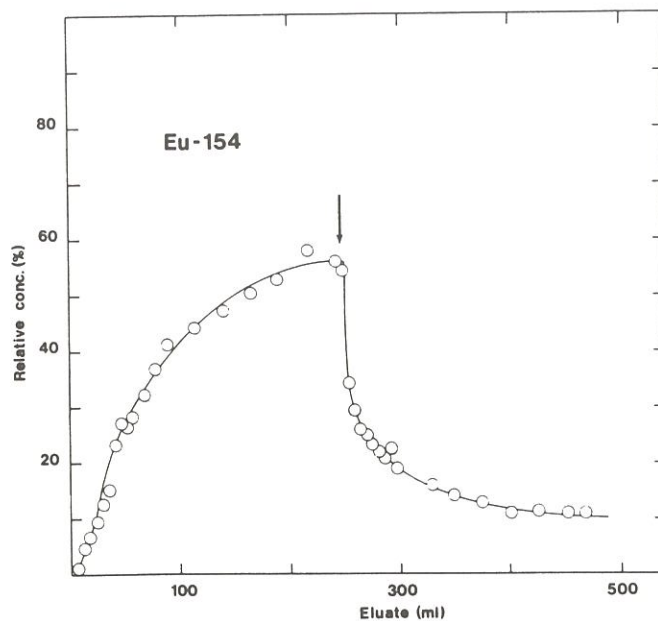
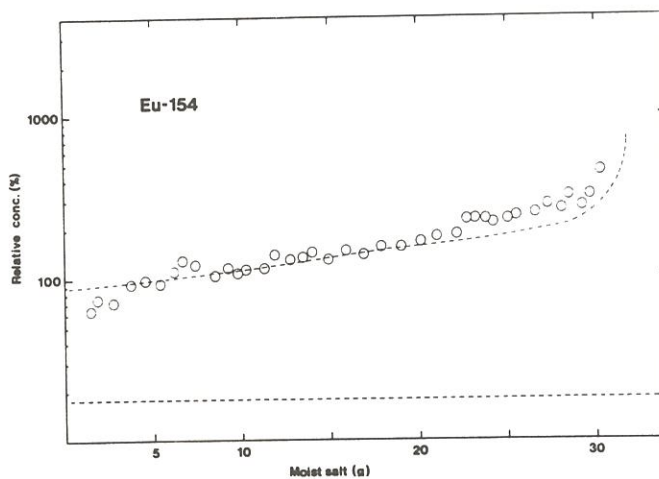*Fig. 7*: Eu-154 elution curve for sorption/desorption experiment.

Fig. 8: Eu-154 concentration profile through the column following sorption/desorption experiment. (dotted line corresponds to "normal" sorption).

4. IODINATION OF HUMIC ACIDS

In the presence of lactoperoxidase (LP) and hydrogen peroxide, humic acids (HA) dissolved in an acetate buffer (pH 5) reacted readily with iodide. Analysis of the HA by liquid chromatography (Fig. 9) demonstrated that iodine was incorporated into all molecular size fractions of the HA. Hence, the reaction products can be described as iodinated humic acids.

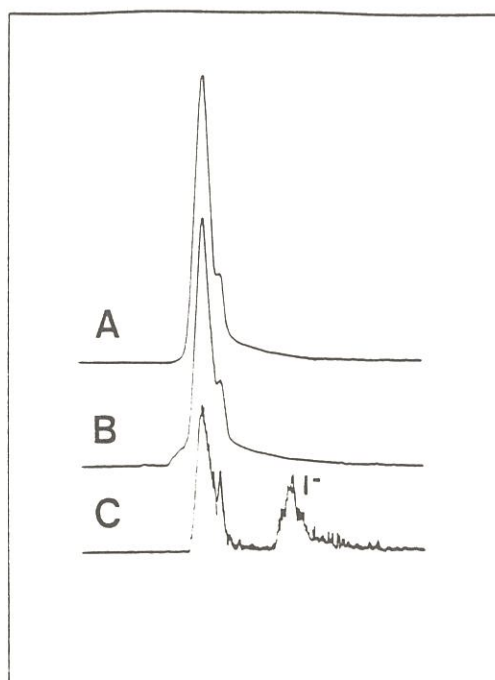*Fig. 9*: Chromatographic trace of A: humic acid prior to iodination (UV detection), B: humic acid after iodination (^{131}I detection).
Initial concentrations: humic acid: 0.1 g/l; iodide: 30 µM; hydrogen peroxide: 200 µM; lactoperoxidase: 10 µg/ml. Reaction time: 20 min.

5. IODINATION OF PHENOL

The importance of the application of different isotopes, and, hence, radiochromatography, in speciation and mechanistic studies can be illustrated by an investigation of the iodination of phenol in aqueous solutions (Christiansen et al., 1990)

The direct iodination of phenol (1) by aqueous I_2 turned, not unexpected out to give five different iodophenols: 2-iodophenol (2), 4-iodophenol (3), 2,6-diiodophenol (4), 2,4-diiodophenol (5) and 2,4,6-triiodophenol (6). The single compounds were identified by comparison with authentic compounds.
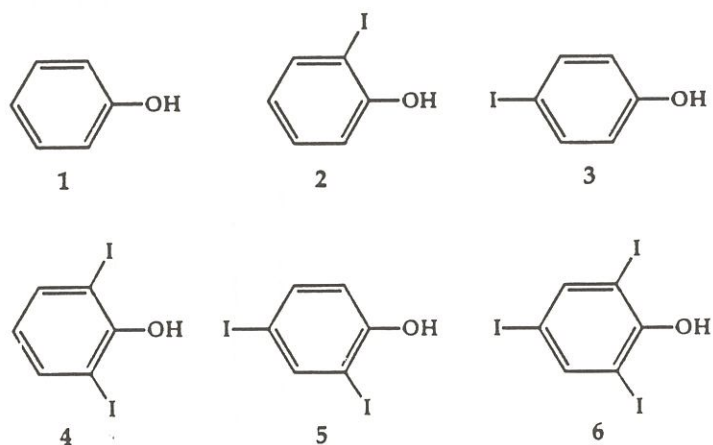
HPLC analysis of the products from the enzymatically mediated iodination of phenol showed no discrepancies compared to that originating from the direct iodination. Enzymatic iodination of phenol using ^{131}I and ^{14}C-phenol, respectively, showed ^{131}I and ^{14}C labeling at the same five positions in the HPLC trace, strongly indicating that the same 5 iodophenols were produced (fig. 10 and 11).
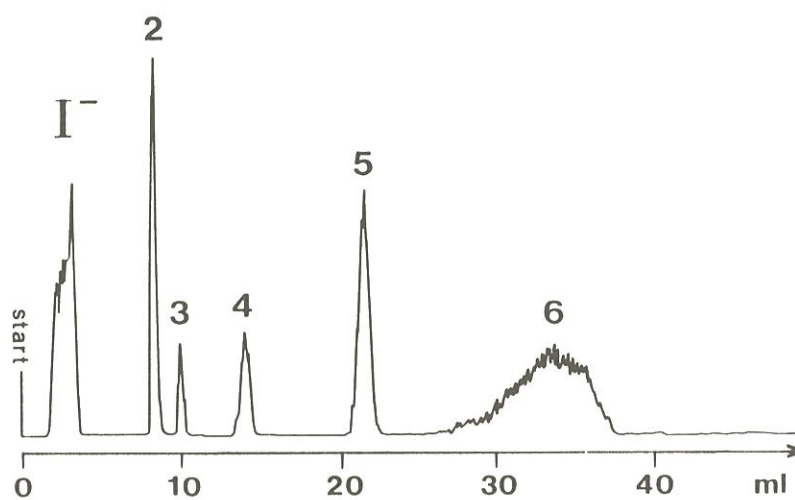
Fig. 10: HPLC trace of the product from enzymatic iodination of phenol using ^{131}I, detecting the radioactivity signal.

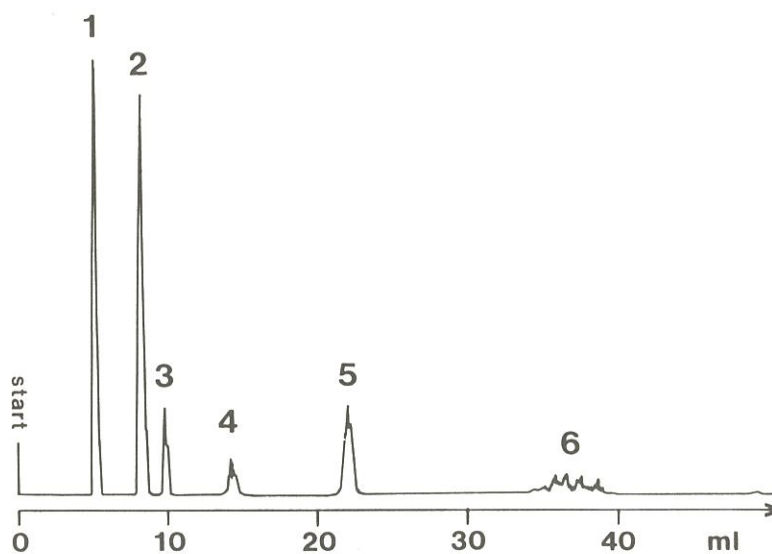*Fig. 11*: HPLC trace of the product from enzymatic iodination of phenol using ^{14}C- phenol, detecting the radioactivity signal.

# 6. ENZYMATICALLY MEDIATED INCOR-PORATION OF PHENOL IN HUMIC ACIDS

The increasing production and consumption of chemicals has increased the risk of percolation of organic compounds and, hence, og the possible polution of fresh-water recipients, e.g. resulting in contamination of drinking water. A significant amount of these organic compounds as well as the posible decomposition products are of phenolic nature.

It has been argued (Stevenson, 1972, 1982, Schnitzer 1981, Varadachari and Ghosk 1984) that humic substances are formed by polymerization of simple monomeric species, such as hydroxy phenols, originating from the decomposition of plant components. Thus, it appears reasonable to assume that other compounds of phenolic nature can be incorporated into an existing humic acid structure. Recently Sakar et al. reported on the enzymatic coupling of 2,4-dichlorophenol to fulvic acids (Sakar et al., 1988).

It seems reasonable to assume that incorporation of extra phenol moieties in the humic acid structure will lead to a material with humic acid characteristics comparable to those of the original humic acid.

Interaction between phenol (spiked with ^{14}C-phenol) and humic acids in solution in the presence of enzymes of the peroxidase group and hydrogen peroxide apparently gives rise to incorporation of phenol moieties in the humic structure. (Lassen et al., 1991b). In Fig. 12 the chromatografic trace (UV-detection) of the humic acid solution before addition of the other reactants (i.e. phenol, horseradish peroxidase and hydrogen peroxide) and that

of the resulting reaction mixture are depicted together with the radioactivity trace corresponding to the latter. Unambiguously, the latter trace demonstrates the incorporation of ^{14}C-phenol in the humic acids.
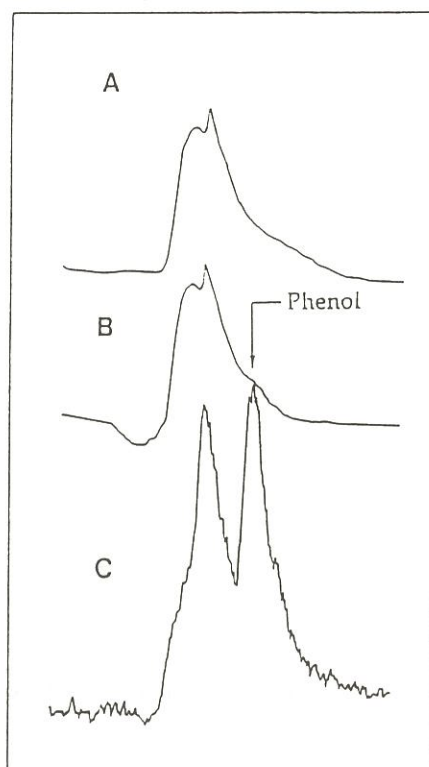
Fig. 12: Chromatografic traces of A (UV-detection): humic acid solution before reacting reaction, B (UV-detection): the resulting reaction, B (UV-detection) and C (radioactivity-detection): the resulting mixture [initial concentrations: humic acid: 935 mg/L, phenol: 5×10^{-4} M, horseradish peroxidase: 1 eu/mL, hydrogen peroxide: 1.6×10^{-3} M, pH 5.5;]

7. CONCLUSION

In sum it can be concluded that radiochromatography appears as a facile experimental technique to determine important physico-chemical properties, as permability, porosity, dispersion-, diffusion-, and sorption characteristics for geological material.

In addition the technique constitute as a rapid method to evaluate the possible influence of changes in ground water composition on the migration behaviour of radionuclides.

Conventional radiochromatography, i.e. liquid chromatography with radioactivity detection, appears as an effective tool in speciation and mechanistic studies of environmental importance.

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