ROLE OF ORGANIC MATERIALS IN A WATER COURSE
WITH RESPECT TO RADIOACTIVE CONTAMINATION
METHOD OF EXTRACTION, FRACTIONATION AND IDENTIFICATION
OF ORGANIC CONSTITUENTS BY SPECTROSCOPY
AND THEIR AFFINITY TO RADIONUCLIDES

(Role de la materie organique d'un cours d'eau vis-a-vis de la
pollution radioactive - methodes d'extraction, de fractionnement et d'identification des constituant organizes par
spectroscopie et leur affinitie vis-a-vis des radionucleides)

By

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Introduction

The significant binding of radioactive contaminants discharged by various nuclear facilities in the organic constituents of a watercourse requires a knowledge of these organic substances as well as their interaction with respect to radionuclides.

A large number of analytical methods have been used to determine the principal chemical characteristics of the organic constituents of soil and sediments as well as the reactions of these substances with other molecules of different nature.

Spectroscopic methods are most frequently used (IR, UV—visible, fluorescence—NMR, mass spectrometry, EPR, x-ray).

However, other analytical tools are also utilized, such as pH-meters, conductimetry, colorimetry, electron microscopy, etc.

However, no author has reported the nuclear technique's which we have utilized; these consist of the complexing of certain radionuclides with a soil or sediment and a study of the organic substances extracted from the soil or a sediment and then fractionated, by means of these techniques. The radioactivity measured in this manner thus is an additional method of investigation to detect the presence of certain substances.

PART I
Nuclear Methods Utilized: Identification of Organic Fractions Forming Strong Bonds with Radionuclides

I. Description of Methods Utilized;
Choice of Materials Utilized

I.1 Method of Extraction with 0.1 N Sodium Hydroxide

A certain quantity of the sediments of the Rhône River is agitated in the water of the Rhône in the presence of selected radionuclides. Settling, followed by filtration, allows elimination of the Rhône water, leaving the sediment which has bound a certain quantity of radionuclides.

Thus contaminated, this sediment is successively agitated with 0.1 N hydrochloric acid in order to remove the carbonates from the sediment, and--after settling and filtration--it is shaken with 0.1 N sodium hydroxide in order to extract the organic materials.
This extraction method with sodium hydroxide must be repeated as many times as necessary (Kononova, 1966). The extraction procedure is shown in Table I.

1.2 Extraction with Ion Exchange Resins

The same quantity of Rhône sediments, water and radionuclides are subjected to the same initial treatment as before in order to obtain the contamination of the sediment.

This sediment is then agitated first with a resin in H\(^+\) form (Relite CFS), intended to release the carbonates from the sediment, and then with a resin in Na\(^+\) form (Relite CFS) in order to eliminate the organic components from the sediment.

Extraction with Na\(^+\) resin is repeated as many times as that performed with 0.1 N sodium hydroxide in the preceding method, in order to obtain a good comparison of the two extraction methods utilized.

The extraction procedure is given in Table II.

1.3 Fractionation of Organic Material

The fractionation of the mixture of solutions extracted with 0.1 N sodium hydroxide and with ion exchange resins should give a more accurate idea of the molecular weight distribution of the organic substances than that obtained by a study of each separate extraction solution. The mixture of extraction solutions obtained with 0.1 N sodium hydroxide and with ion exchange resins is concentrated in a ratio of 10 by a rotary evaporator at an average temperature of 40°C and is then brought to neutral pH by addition of HCl. Finally, a certain quantity of NaCl is dissolved so that a solution with a normality of 0.2 NaCl is obtained.

1.3.1 Fractionation by ultrafiltration. Up until now, ultrafiltration (Prudhomme, 1957; Van Oss, 1970; Michaels, 1968) was used to fractionate and purify certain molecular species, particularly proteins; however, it does not seem to have been the subject of a study dealing with organic constituents extracted from a soil or sediment.

The method utilized here required the following equipment: An ultrafiltration cell (Sartorius, SM 16 244) placed under pressure with bottled nitrogen (the operating pressure of the cell is selected at between 1 and 3 bars depending on the ultrafiltration membrane utilized).
Ultrafiltration membranes with retention ranges corresponding to molecular weights of about 1000 (Millipore, Pellicon PSAC), 25,000 (Millipore, Pellicon, PSED) and 70,000 (Sartorius, SM 117 33).

A membrane with a retention of about 500 consisting of dialysis cellophane and calibrated with certain color reagents, the concentrations of which are measured by their absorption in the visible spectrum.

I.3.2 Fractionation by gel chromatography. This method has been used by a number of authors, particularly by Butler, Ladd (1969); Ladd (1969); Lindquist (1967); Ferrari, Dell'Agnola (1963); Posner (1963); Mehta et al. (1963).

The chromatographic columns used are of glass (I.D. = 2.4 cm, h = 100 cm). The gel packing has a height of about 75 cm. The void space is determined by elution of Dextran Blue 2000 which is completely eliminated from the gels utilized. The elution rate (0.25-0.4 ml/min) is assured by a Buchler micro-roller pump.

A micro-fraction collector allows sampling of fractions of 10 ml each directly in the tubes used for counting in order to avoid a possible activity loss.

The eluant utilized is 0.2 M NaCl and the sample volume of organic material loaded on the gel surface by a sample applicator is 10 ml. The gels used are "Sephadex" G 10, G 15, G 50 and G 100, the technical data of which were furnished by the manufacturer (Pharmacia Fine Chemicals, Uppsala, Sweden) or by various authors, for example, Determann (1969); Fischer (1969).

I.4 Methods of Investigation

I.4.1 Methods for radioactivity measurement of the samples. All of the radionuclides utilized are $\gamma$-emitters. The radioactivity measurement in samples of the collected organic solutions is carried out with an Intertechnique SA 40 B 400-channel pulse-height analyzer together with a NaI(Tl) 3" x 3" well-scintillation counter placed into a lead chamber of 10 cm wall thickness.

The calculation of the activity of each of the radionuclides from the spectrum recorded on punched tape is performed by an IBM 360 computer.
1.4.2 Spectroscopic method. The direct measurement of the optical densities at 280 and 450 m\(\mu\) of the samples obtained by fractionation is performed with a Jobin-Yvon single-beam spectrophotometer.

The absorption spectra in the UV- and the visible region are also recorded with the same spectrophotometer for a number of fractions. The cuvettes utilized have an optical path of 5 mm for UV and 40 mm for the visible region (when absorption is too weak to be measured with a 5 mm cuvette).

1.5 Choice of Materials Utilized

\textbf{Sediments.} These sediments were taken from the Rhône 5 km upstream from the city of Arles in the settling chamber of the Rhône sampling station from the irrigation canals of the Compagnie Nationale d'Aménagement du Bas Rhône - Languedoc.

The sediments were selected as a function of their high affinity for radionuclides, accessibility of the sampling point and previous studies (Picat, Grauby, 1969).

The water was also taken at the above-specified place.

\textbf{Radionuclides.} The following list of radionuclides was investigated: \(^{141}\text{Ce}, ^{133}\text{Ba}, ^{106}\text{Ru}, ^{137}\text{Cs}, ^{54}\text{Mn}, ^{65}\text{Zn}, ^{60}\text{Co}.\) This choice was based on the following main reasons:

1. The presence of these radionuclides in the waste effluents of existing or future nuclear facilities, processing plants and reactors (enriched-uranium reactor system).

2. The hazard which they represent for the population with consideration of their physicochemical characteristics, their assimilation by man and their radioactive half-life.

3. The characteristics of their \(\gamma\)-emission spectrum permitting their mixing at the same time as a sufficiently simple distinction, so that analysis of the spectra by the least squares method obtains the highest possible reliability and accuracy.

II. Results

\textbf{II.1 Extraction Results}

Figs. 1 and 2 show the cumulative percentages\(^1\) of extracted radionuclides bound to organic material as a function of time and the successive extractions. Furthermore, the cumulative optical
density curves at 450 and 280 m\(\mu\) were plotted as a function of time, giving an idea of the quantity of organic material extracted and its color with time.

II.2 Fractionation Results

Table III shows the percentages\(^2\) of the radionuclides bound to the organic molecules with molecular weights higher than:

\[ x = 500, 1000, 25,000, 70,000 \text{ for ultrafiltration} \]

and

\[ x = 700, 1500, 10,000, 100,000 \text{ for gel chromatography}. \]

This table also lists the percentages\(^3\) of the optical densities at 450 and 280 m\(\mu\) for the different fractions of organic material obtained.

In the case of gel chromatography, the radioactivity and optical densities of the fractions obtained were calculated by planimetry of the elution curves (see Figs. 4-7).

The activity and optical density percentages are obtained by the ratio between the area \(S_1\) of the retained fraction to the sum of areas \(S_1 + S_2\) of the total retained and retarded fractions (see Fig. 3). The values of \(^{141}\)Ce and \(^{65}\)Zn could be determined. Figs. 4-7 show the elution curves corresponding to fractionation on Sephadex gels \(G_{10}, G_{15}, G_{50}, G_{100}\), which have retention ranges for molecular weights of 700, 1500, 10,000 and 100,000, respectively.

The optical densities at 280 and 450 m\(\mu\) as well as the activities of the investigated radionuclides were plotted on the same logarithmic scale as a function of the elution volumes expressed in ml.

Table IV shows the optical densities at 450 and 280 m\(\mu\) as well as their K-ratio for organic molecules with molecular weights of less than 500, 1000, 25,000 and 70,000 for ultrafiltration and 700, 1500, 10,000 and 100,000 for gel chromatography.

Finally, Table V furnishes the activity percentages, the mean activity percentages, the optical densities at 450 and 280 m\(\mu\) as well as their K-ratio for the peaks present on the elution curves of fractions retarded by gels \(G_{10}\) and \(G_{15}\).

The first value of each column represents the percentage with respect to the \(S_2\) values.

The second value represents the percentage with respect to the \(S_1 + S_2\) values.
The percentages were also obtained by the above-described planimetry method.

The elution volumes of these fractions (I to IV) were recorded in parentheses on the same lines as the results of \( G_{10} \) and \( G_{15} \).

Footnotes

1) The percentage of radioactivity associated with each extraction is expressed by the ratio of the activity of the extraction solution to the activity of the sediment after contamination multiplied by 100.

2) The percentage of radioactivity bound to molecules with molecular weights higher than a value \( X \) is expressed by the ratio of the activity of the fraction of organic material higher than \( X \) to the total activity of the organic material after extraction, multiplied by 100.

3) The optical density percentages are expressed by the ratio of optical densities of the isolated fractions to the optical densities of the extraction solution, multiplied by 100.

III. Discussion of Results

III.1 Comparison of Extraction Methods

A comparison of the curves showing the cumulative percentages of radionuclides bound to organic material with respect to their total activities contained in the sediment as a function of the extraction time give an advantage to the extraction method with 0.1 N sodium hydroxide compared to the ion exchange method, except for the particular case of cesium. Furthermore, a number of authors (De Borger, 1967; Kononova, 1966) believe that the sodium hydroxide method extracts more organic material than the other normally used methods.

In contrast, a comparison of the optical density curves at 280 and 450 m\( \mu \) seems to indicate that the organic material is extracted better by exchange resins than by 0.1 N sodium hydroxide. These findings appear to be explainable in two ways:

1. 0.1 N sodium hydroxide would extract a fraction of organic material bound to the inorganic material in smaller proportion compared to that obtained during resin extraction.

2. 0.1 N sodium hydroxide might extract a group of activity-carrying organic substances which are not extracted by the resins and which would produce only a weak absorption at the wavelength utilized (280 and 450 m\( \mu \)).
In order to clarify this point, studies are presently being carried out concerning purification methods for the extracted organic material.

On the other hand, a comparison of the K-values, representing the optical density ratio at 450 and 280 m\textmu, indicates that the organic material extracted by the resins is much more colored than that extracted by 0.1 N sodium hydroxide.

Furthermore, successive extractions in the case of sodium hydroxide as well as with ion exchange resins reveal an increase of the K-value up to the 4th cycle and then a decrease to the fifth. On the basis of our subsequent considerations, this proves that the molecular weights extracted in successive cycles increase up to the fourth; this seems logical, since the larger molecules solubilize with greater difficulty than the small ones.

Finally, examination of the percentages of radionuclides extracted with the organic material reveals different sequences. With both extraction methods, the extracted ruthenium has the highest percentage.

For the other investigated elements, the different extraction procedures may explain differences in the order of the extracted percentages. In fact, the extraction method with sodium hydroxide does not follow the same laws as those which govern the extraction mechanism with ion exchange resins in which ion selectivity laws (i.e. primarily the ionic charges and hydrated volumes) are involved (Samuelson, 1967; Tremillon, 1965; Reichenberg, 1966).

For the majority of cations, the difference in the order of classification are minimal, except for $^{141}\text{Ce}$ which is located in sixth position in the percentages extracted with sodium hydroxide and in the second position in the case of extraction with ion exchange resins.

III.2 Comparison of Fractionation Methods

The two methods agree in indicating that the largest part of the organic constituents extracted from the sediment consists of very small molecules, i.e. molecular weights lower than 500-700.

Studies presently in progress have the purpose to determine whether these results are close to reality in the sediment or if the extraction methods utilized for this investigation have somewhat modified the organic material during its extraction by degradation of certain macromolecules (Flaig, 1970).
On the other hand, close examination of the comparative table of fractionation results shows certain significant differences between the two methods, which have several important reasons:

a) Interactions between the complex of organic material-radiouclides and the gels, on one hand, and the membranes, on the other, certainly are of different nature. Certain authors (Determann, 1969; Lindquist, 1967), for example, have observed an interaction between the Sephadex gels and certain molecules, for example, those of a benzene or aromatic character. Furthermore, (Butler and Ladd, 1969) found a higher absorption with gel $G_{15}$.

b) The manufacturers determined the retentions of membranes with a cut-off of 25,000 and 70,000 using solutions of certain proteins, while those of other membranes and Sephadex gels were not made with the same molecular species.

c) The determination of the retained and the retarded fraction of a gel can only be established with a certain error because it is impossible to find a well-defined limit between these two fractions on an elution curve.

On the other hand, the results of the optical density values (O.D.) at 450 and 280 m$\mu$ as well as their ratio $K = \text{O.D. (450)}/\text{O.D. (280)}$ (see Table IV) show the following:

For fractionation by gel chromatography we have found no relation between the molecular weights and the color of the fractionated organic substances, as was true for Butler and Ladd (1969) who fractionated humic acids on Sephadex gels. The interaction between the gel matrix and the organic-radiouclide complexes seems to be the reason for this.

In fact, we observed sometimes significant binding of the radionuclides by the gels utilized, particularly by gel $G_{15}$. We can estimate that most, if not all, of the bound radionuclides originate from the retained fraction which passes into the gel first. For this reason we have recalculated the percentages of the radionuclides of the retained fractions, using for the new value of the retained fraction the old value of the latter added to the value bound on the gel for each radionuclide. The new values of the retained fractions are listed in Table III below the old ones for the corresponding cases.
It is evident that the mean percentages obtained in this case are closer to the percentages of the optical densities obtained by chromatography. $G_{15}$ gel seems to have too high a value attributable to its significant interaction with the investigated substances.

For $G_{100}$, binding probably is not due only to the retained fraction which is very small and cannot saturate the gel with radionuclides; it may also be due to the retarded fraction. Consequently, the estimated value of the retained fraction is higher.

In any case, the real values should fall between the old and the new ones obtained.

For fractionation by ultrafiltration, there is a proportionality between the color (K-values) and the molecular weights: the most colored molecules have the highest weights.

On the other hand, the percentages of the optical densities at 280 μ and the mean percentages of the radionuclides are similar for the same ultrafiltration membrane. Thus, 280 μ is the mean absorption value of nonconjugated carbonyl fractions (Scott, 1964). This shows that radionuclide binding by the organic material takes place primarily via the carbonyl groups.

On the other hand, the above considerations mean that the interactions between the investigated substances and the ultrafiltration membranes utilized are not very important in contrast to the gels utilized for chromatography.

**IV. Conclusion**

The above-described fractionation methods permit us to obtain some certainty concerning the interaction of radionuclides with the organic material extracted from a sediment.

It is evident that a spectroscopic, chemical study and other fractionation methods are necessary to obtain better knowledge of this interaction. These analytical methods are described in Part II.
PART II

Spectroscopic Methods Utilized: Identification of Certain Substances Which Strongly Bind Radionuclides

I. Spectrophotometric Analysis of Certain Fractions Obtained by Gel Chromatography

The absorption spectra of unfractionated organic material extracted from a soil or sediment show a monotonic course both in the UV and the visible regions (Kononova, 1966) due to the large number of different molecular groups constituting these organic materials. Fractionation is therefore necessary to reveal some of the chemical characteristics and sometimes even certain molecules.

Thus, Sato and Kumada (1967) found certain polycyclic hydrocarbons.

On the other hand, Schnitzer and Skinner (1968), using Sephadex gel chromatography, obtained a spectrum with well-defined peaks only for a single one of their fractions.

Finally, Ziechman (1964) obtained a few well-defined absorption bands by solvent extraction. On our part, spectroscopic study of the fractions representing the principal peaks of the elution curves from gels G\textsubscript{10} and G\textsubscript{15} furnished interesting results. Each investigated fraction has very specific spectral characteristics, although they are sometimes similar.

In fact, all fractions investigated reveal the presence of polycyclic hydrocarbon derivatives as well as occasionally molecules of porphyrin nature.

In our study we compared particularly our own results with those of Hodgson et al. (1967).

Fractions of Gel G\textsubscript{10}

The UV-spectra of fractions I and II of Table V indicate the presence of polycyclic quinones with 3 or 4 benzene rings (absorption bands at 250, 282, 302 and 360 \textmu m), while the optical densities decrease markedly at the beginning of the visible region.

In the visible region, these fractions would seem to indicate the presence of a certain number of porphyrins; in particular:

Free etioporphyrins with molecular weights of between 400 and 500 (Thomas and Blumer, 1964).
Etioporphyrins complexed with V and Ni (Gransch and Eisma, 1966; Champlin and Dunning, 1958; Hodgson and Baker, 1967; Millson et al., 1966).

Finally, chlorines (Hodgson and Baker, 1967; Falk, 1964; Manskaya and Drozdova, 1968).

Furthermore, a strong band at 485 μm as well as other less well-defined bands between 400 and 500 μm seem to indicate the presence of carotenoids (Trichet, 1970; Dean, 1963; Scott, 1964; Zechmeister and Polgar, 1943).

It is evidently difficult to make quantitative determinations for the respective quantities of polycyclic quinones, porphyrins and carotenoids present in these fractions.

Fractions III and IV also show characteristic spectra of polycyclic quinones in the UV region. In the visible region no absorption suggests a significant quantity of porphyrins. However, a fairly considerable amount of carotenoids can be observed.

**Fractions of Gel G₁₅**

Fraction I reveals the presence of polycyclic quinones as well as probably metal-porphyrins (Hodgson et al., 1968).

Fraction II seems to contain polycyclic quinones, less visible than in the case of Fraction I, and porphyrins of the type ET₁₀ and PHYLLON in smaller quantity than in Fraction I.

As in the case of gel G₁₀, a strong band at 485 μm as well as other less well-defined ones might indicate substances with a carotenoid structure (Trichet, 1970; Dean, 1963; Scott, 1964).

Fractions III and IV contain anthraquinones and other polycyclic quinones as well as definitely quinone perylenes (molecular weights of about 250). In fact, the peaks at 362, 383, 400-415 μm and the peak of maximum intensity in this region at 436 μm resemble those reported by Hodgson et al. (1968) in the case of perylene: 362, 382, 408, 428 and 434 μm (max.).

On the other hand, a notable absorption up to 500 μm suggests the probable presence of carotenoids.

A considerable quantity of Ba is bound to fraction III. Fig. 8 shows the spectra obtained for Fractions I-IV of gel G₁₅.
Fraction of Molecular Weights Below 500

This fraction was obtained by ultrafiltration (see above).

In view of the broad range of molecules constituting this fraction, the spectrum obtained is relatively monotonic and resembles those obtained for the unfractonated organic material. Nevertheless, a band can be seen at about 250 μm, several poorly defined bands in the 500-600 μm region as well as a sharper band at about 620 μm. The bands of the visible region suggest the presence of porphyrins (particularly etioporphyrins with weights of 400-500 according to the results of Thomas and Blumer (1964).

Examination of Table V permits us to state that fractions II from gels G₁₀ and G₁₅ are those which bound the largest quantity of radionuclides. These fractions seem to contain primarily the polycyclic quinones, porphyrins as well as carotenoids. On the other hand, these fractions are also those which have the smallest K-values: 0.450/0.280. This means that these fractions have very high optical densities at 280 μm. However, 280 μm is the mean absorption value of nonconjugated aldehydes and ketones (Scott, 1964). Finally, examination of the spectra obtained reveals strong absorptions at about 220-250 μm in the case of fractions II from gels G₁₀ and G₁₅ compared to the other fractions. These absorptions indicate the presence of conjugated carbonyl functions (Scott, 1964). Finally, the quinones absorb at between 240 and 290 μm according to Scott (1964).

These findings demonstrate that the most active fractions of the investigated organic substances with regard to radionuclides have a large number of conjugated or nonconjugated carbonyl functions which are responsible for radionuclide binding. Finally, it is possible that the presence of porphyrins in the investigated fractions plays a fairly considerable role in the binding of radionuclides.

II. Use of Reductive Chemical Methods and Spectrophotometry: Identification of Certain Molecular Groups

The above-described spectroscopic study had shown the presence of well-defined molecular groups in the fractions obtained by chromatography on gels G₁₀ and G₁₅, suggesting that these groups can be fractionated in turn. Consequently, solvent extractions were performed with the fractions described above in the following manner:
A water-ethanol solution \((50\%)\) containing the substances fractionated on gels \(G_{10}\) and \(G_{15}\) was vigorously agitated first with toluene, then with petroleum ether and hexane for reaction periods of 24 h. For each extraction, a spectroscopic study shows that the extracts are primarily polycyclic quinones, generally related to anthraquinone and phenanthrene quinone, containing phenol fractions (hydroxyanthraquinones). In fact, the absorption frequencies between 250 and 300, at 325 and between 400 and 450 are characteristic of these quinones and the strong absorption at about 270-280 \(\text{nm}\) seems to indicate \(\text{OH}\) groups (Scott, 1964).

Fig. 9 shows the absorption spectra of the substances extracted from the first fraction of gel \(G_{10}\) (Table V) by means of the above three solvents.

In order to obtain some certainty on this point, several reducing methods were used for the fractions obtained by the various above-cited extractions. By these methods, the polycyclic quinones should convert into the corresponding polycyclic hydrocarbons.

The reduction method with zinc powder, pyridine and acetic acid described by Clar (1964) was modified as follows:

First of all, pyridine was replaced by the solvent used for the extraction (toluene, petroleum ether, hexane). This solvent is mixed with acetic acid \((80\%)\) in the presence of zinc powder and then brought to boiling.

In a first technique, boiling was performed without refluxing. It was also carried out at the same temperature with the reference solvent mixed with acetic acid \((80\%)\). This reference solvent is intended for spectroscopic study.

In a second procedure, boiling was carried out with refluxing. In this case, the solvent retains the same composition for the period of boiling.

Finally, a third technique (fusion with zinc powder) described by Hansen and Schnitzer (1969) and other authors was slightly modified with the use of toluene instead of hexane as a solvent.

All of the methods described, although sometimes furnishing a rather small yield, show a very marked reduction of polycyclic quinones into the corresponding hydrocarbons.

Figs. 10, 11 and 12 show the respective spectra obtained by the above methods in the case of toluene. In the other cases the spectra had similar shapes.
Boiling With Zinc Powder Without Refluxing (Fig. 10)

Curves 1, 2 and 3 show the respective spectrum of the unreduced fraction, the spectrum of the same fraction after 5 h 30 min reaction, and the spectrum of the same fraction after 10 h of reaction. The three curves have three isobestic peaks at 340, 415 and 525 μm due to the change of concentration of the acetic acid solution, i.e. a pH change of the solution.

The significant variation of absorption at about 280 μm may be due to a phenomenon other than quinone reduction (solvent effect, etc.), so that it is advisable not to draw a conclusion on the spectroscopic results obtained in this case, even though the increase of absorption between 340 and 380 μm (Clar, 1964; Scott, 1964) suggests a reduction of anthraquinones and of phenanthrene quinones.

Boiling in the Presence of Zinc Powder

With Refluxing (Fig. 11)

The curves of Fig. 11 reveal the reduction of the polycyclic quinones into polycyclic hydrocarbons with a structure related to that of anthracene, phenanthrene and 3,4-benzopyrene: absorptions at about 330-380 μm (Clar, 1964).

Spectra 1-4 show the respective substances investigated without reduction and after reduction for 3 h, one night and three days of reaction; in contrast to the case of reduction without refluxing, we observe no change in absorption at 280 μm.

Fusion With Zinc Powder (Fig. 12)

Spectrum 1 of the unreduced quinones indicates the presence of anthraquinones (325 μm), phenanthrene quinones (362, 388 μm) and hydroxyanthraquinones (460 μm) (Clar, 1964; Scott, 1964; Hirayama, 1967).

Spectrum 2 obtained after reduction reveals polycyclic hydrocarbons in larger quantity than in the preceding method. These results suggest the presence of 3,4-benzopyrene (284, 296, 330, 347 μm), 1,2-benzopyrene (331, 350-360 μm), pyrene (292, 333, 351, 356 μm), perylene (382, 434 μm), phenanthrene (293, 330, 346 μm) and anthracene (357, 375 μm).

The above-described two reduction methods, particularly the latter, indicate that the most reactive groups of substances consist of polycyclic quinones with 3-5 benzene rings (C_{14}-C_{20}) for the investigated fractions.
III. Utilization of Thin-Layer Chromatography and Spectrophotometry: Identification of Certain Particularly Active Molecules for Radionuclides

The most detailed study of the molecular groups found in the preceding section can be made with the use of thin-layer chromatography techniques.

### III.1 Experimental Technique

**Choice of solvent system.** Experimental studies using the theoretical data furnished by a study of elutropic series defined in certain books (Browning, 1971; Randerath, 1971; Vernin, 1970; Bobbitt, 1963) permitted us to obtain an eluent forming a system of polar and non-polar solvents as necessary to fractionate the above-defined polycyclic molecules. The eluent composition was determined to be as follows:

- Toluene   2/30
- Acetic acid 8/30
- Alcohol 1/3
- Hexane 1/3

**Choice of adsorbent.** In order to obtain reliable results by thin-layer chromatography, three adsorbents were utilized:

- Polycarbonate adsorbent (Kodak K511V)
- Silica gel adsorbent (Schleicher and Schüll F1 500)
- Cellulose adsorbent (Schleicher and Schüll F1 440)

All chromatographic plates used were precoated on a plastic support. It is thus easily possible to cut the different groups of substances obtained after chromatography with scissors and to dissolve each group thus separated in a suitable solvent more easily.

**Sample application.** The reduction methods by boiling with and without refluxing were compared in the case of the polycarbonate adsorbent to obtain greater certainty concerning the results obtained. The results obtained in the case of boiling without refluxing had led to very similar conclusions for the three adsorbents, so that only the polycarbonate adsorbent was investigated for the substances obtained by boiling with refluxing.

Consequently, the applications made by a microsyringe (between about 20 and 60 µl) originated from the solution obtained by reduction with zinc powder by boiling without refluxing, on one hand, and the solution obtained by boiling with refluxing, on the other.
Technique utilized. The ascending chromatographic technique with a standard tank was employed. The Rf corresponding to each separated compound was calculated.

UV-spectroscopic detection. For each Rf obtained, the spectra were recorded in the UV region and at the start of the visible region between 215 and 410 m. In the visible region no significant absorption could be observed. The substances obtained after fractionation were dissolved in a solvent (ethyl alcohol) as mentioned above.

III.2 Results

Figs. 13, 14 and 15 show the spectra obtained for each Rf for the polycarbonate, silica gel and cellulose adsorbents in the case of substances reduced by boiling with zinc powder without refluxing.

Tables VI, VII and VIII furnish the absorption frequency assignments of the spectra represented in Figs. 13, 14 and 15, respectively, as well as the identification of the corresponding substances.

Fig. 16 and Table IX show the same results obtained for starting substances which were reduced with zinc powder by boiling without refluxing [sic].

III.3 Discussion of Results

Analysis of Tables VI, VII and VIII shows that for each Rf value, the identified substances are practically the same regardless of the adsorbent utilized. More precisely, it may be noted that the number of aromatic rings decreases with increasing Rf, a decrease going from C20 to C8. In the case of Table IX, the same variation of Rf as a function of the number of rings is noted.

On the other hand, in all investigated cases, the spectra obtained show a progression in the 250-320 m zone going from a double group of absorption bands for the small Rf values to a single and broad absorption band for large Rf values. According to Clar (1964), this results from the shift of the β- and p-bands toward the red region (bathochromic effect) when the number of rings increases.

In fact, the absorptions corresponding to the p-bands vary progressively from 270 to 290 m when Rf decreases, while those corresponding to the β-bands vary from 220 to 250. These interpretations are more evident when Fig. 17 is examined.
TLC study permitted us to demonstrate the presence of a series of polycyclic hydrocarbons derived from the corresponding quinones. Hansen and Schnitzer (1969) obtained identical substances by distillation and fusion with zinc powder.

Finally, considering the optical density values of the different spectra obtained, we observed—regardless of the adsorbent utilized—that the absorptions were highest for the largest Rf values, i.e. for Rf = 0.88, 0.93 and 0.92, respectively, with the polycarbonate, silica gel and cellulose adsorbents.

This means that the naphthoquinones and anthraquinones represent the major part of the fractionated quinones.


Fig. 18 shows the Kekule plots for the chemical substances described above.

Fig. 19 permits a comparison of certain spectra obtained with the spectra of the pure substances derived from the studies of Clar (1964) and Scott (1964).

Footnotes

1) \( Rf = \frac{\text{Distance of origin-compound}}{\text{Distance of origin-solvent front}} \)

2) Classical designation of the principal bands of aromatic compounds.

IV. Conclusion

Chromatographic methods combined with solvent extractions prove to be effective for the fractionation of natural substances, particularly in the case of an organic material of a sediment.

On the other hand, analytical methods by spectroscopy in the UV and visible regions permit a reliable identification of certain substances.

The rapid advances in equipment used for chromatography as well as the improvement of the technology of spectrophotometers should permit us to obtain more reliable knowledge of natural substances.
General Conclusion

The important role of organic materials in a waterway with respect to radioactive contamination requires a knowledge of the sorption and desorption of radionuclides by and from these organic materials. This is why the present study had the purpose to compare some of the extraction methods for organic materials present in a sediment and to define the interactions between a number of radionuclides and the extracted organic substances.

To this end, fractionation of the organic material was carried out as a function of molecular weights and an attempt was made to characterize the most reactive substances.

We propose to continue these studies by various possibilities of further refinement:

First, a study dealing with milder extraction methods to avoid or limit possible degradation of the extracted substances.

Then, the application of analytical techniques to the study of organic compounds in solution in water in equilibrium with the sediments should permit a comparison of the results obtained for the organic material of the sediment.

Finally, a refinement of the identification techniques by the use of other methods (NMR, etc.) as well as an attempt at a direct characterization of the quinones involved in complexing, should provide us with better knowledge of the organic substances responsible for binding of radionuclides and other chemicals.

Literature


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