Acta Chemica Scandinavica B 33 (1979) 457—459

Removal of Elemental Sulfur from Environmental Samples

KJELL TJESSEM

Chemical Institute, Realfagbygget, University of Bergen, N-5000 Bergen, Norway

This work describes a new and harmless method for quantitative removal of elemental sulfur from lipids by the cross-linked polystyrene gel Bio-Beads SX-12. The method offers an advantage over the more conventional method with activated copper powder [1] in that polar material and labile compounds can be eluted without being adsorbed or degraded by the gel matrix.

Sediments act like a sink for many environmental compounds including aromatic hydrocarbons and their oxidation products.[2] Because of the believed carcinogenic effect of many of these compounds [3] it is very important to be able to analyze them in order to predict their fate and effect in sediments and corresponding benthos.

Elemental sulfur is present in most unconsolidated recent marine sediments. In the isolation method commonly used for isolation of lipid material from sediments,[4] elemental sulfur is also recovered and often exceeds the small amount of organic constituents. The most common method for removal of free sulfur from sediments prior to analysis by gas chromatography or GLC/MS has been by percolation through a column with activated copper powder.[1] However, many polar compounds are known to interact strongly with activated copper powder,[1] and many compounds formed as a result of metabolism or oxidation are very labile and may be degraded or destroyed in contact with activated copper.

In addition, sediment samples may contain so much elemental sulfur that the normal copper method simply cannot handle it all, and traces of free sulfur can pass through. It is well-known that even trace amounts of elemental sulfur severely interferes with GLC/MS analyses.

0302-4369/79/060457-03$02.50

The aim of this work has been to establish a method for quantitative removal of elemental sulfur and at the same time obtain a quantitative recovery of polar metabolites and oxidation products without any degradation of the compounds.

EXPERIMENTAL

Bio-Beads SX-12, a cross-linked polystyrene gel from Bio-Rad Laboratories with an exclusion volume of about 400 was equilibrated in acetone overnight.

The gel was then packed into a glass column with the dimensions 25.4 × 580 mm and eluted with acetone at a rate of 30 ml h^{-1} using a solvent delivery system 711 from Laboratory Data Control (LDC), Division of Milton Roy Company. The solvents used in this experiment were degassed immediately before use. Both the chromatography step and further handling of the compounds were carried out in a dark room with only red light present in order to minimize photo-oxidation.

After a 12 h equilibration period the following standard compounds and test mixtures were loaded onto the column.

1. 9-Hydroxy-1,2-benzanthracene (12 μg).
2. 8,9-Dihydrodiol-1,2-benzanthracene (15 μg).
3. 1,2-Benzanthracene (20 μg).
4. Free sulfur (0.5 mg) and ^{14}C-1,2-benzanthracene of known activity (~800 cpm) dissolved in a small amount of toluene.
5. A dried sediment sample extracted with a (1:1) mixture of toluene–methanol and spiked with ^{14}C 1,2-benzanthracene of known activity (~850 cpm).

Fractions of 15 ml each were collected from the column and the distribution of the various standard compounds in the different fractions were established by high performance liquid chromatography using a reverse phase Zorbax ODS column and a UV detector operating at 254 nm.

The recovery of the standard compounds and of the radioactive 1,2-benzanthracene was measured by high performance liquid chromatography and scintillation counting, respectively. The radioactive samples were counted in 3 ml Aquasol mixture for 50 min or to 1% error. The counting efficiency was over 90 %.

Elemental sulfur and other material sticking to the gel matrix were eluted from the Bio-Bead gel with toluene. The column was then re-equilibrated with acetone and ready for the next run. The elution speed for toluene was set to 75 ml h^{-1}.

Table 2. Recovery of free sulfur and ^{14}C-1,2-benzanthracene from Bio-Beads SX-12 gel based on scintillation counting and gravimetric determinations on an electrical Cahn balance.

	Sulfur %	^{14}C-1,2-Benzanthracene %
Eluted with acetone	0	98
Eluted with toluene	99	0

RESULTS AND DISCUSSION

Table 1 clearly shows that within experimental errors there is a quantitative recovery of 1,2-benzanthracene and selected metabolites from the Bio-Beads SX-12 gel, and we could not detect any degradation products by high performance liquid chromatography using a Zorbax ODS column. Table 2 also shows that there is a quantitative separation of free sulfur from ^{14}C-1,2-benzanthracene by this method. All acetone fractions were screened for free sulfur using gas chromatography with an electron capture detector. Only trace signals could be observed. No activity above background could be found in the toluene fraction. The same is also true for ^{14}C-1,2-benzanthracene spiked with the extracted sediment sample. No sulfur could be observed in the acetone fractions and no activity could be found in the toluene fraction. On the other hand free sulfur from the sediment sample could be detected in the toluene fraction.

Since this is a chromatographic method we have tried to keep artefact formation to a minimum by excluding light and deoxygenating the solvents. Only organic solvents have been used in the extraction of metabolites from sediment samples,[2] and we have deliberately tried to avoid any acid/alkali extractions. Any opportunity for chemical reactions has therefore been greatly reduced from that accompanying acid/alkali extractions or liquid chromatography with activated copper powder because the constituents now only contact organic solvents and the relatively inert gel matrix.

Bio-Beads SX-12 with acetone as the mobile phase has been shown to elute aromatic hydrocarbons in order of increasing ring number,[5,6] but the general elution picture has also been shown to depend very much on the substitution.[7] Table 1 also indicates that polar substituted 1,2-benzanthracenes are eluted before their parent aromatic hydrocarbon. This can be ascribed to the gel permeation effect of these substituents with this particular gel and mobile phase.

In addition to offering a complete separation of most organic constituents of interest and free sulfur, the method also serves as a good "clean up" for environmental samples. All

Table 1. Elution behaviour and recovery of 1,2-benzanthracene and metabolites from Bio-Beads SX-12 gel based on high performance liquid chromatography.

Compound	Fraction number	Recovery %
1,2,Benzanthracene	9 – 10	99
9-Hydroxy-1,2-benzanthracene	7 – 8	98
8,9-Dihydrodiol-1,2-benzanthracene	6 – 7	98

Table 3. Recovery of ^{14}C-1,2-benzanthracene spiked with an extracted sediment sample based on scintillation counting.

	Sulfur %	^{14}C-1,2-Benzanthracene %
Eluted with acetone	0	98
Eluted with toluene	–	0

polymetric and high molecular weight materials are being excluded from the run, while isolation of saturated hydrocarbons, aromatic hydrocarbons and polar compounds can be obtained.

REFERENCES

1. Blumer, M. *Anal. Chem. 29* (1957) 1039.
2. Farrington, J. W., Tjessem, K., Lee, R. F. and Hinga, K. *In preparation.*
3. Hoffman, D., Bondinell, W. E. and Wynder, E. L. *Science 183* (1974) 215.
4. Farrington, J. W., Giam, C. S., Harvey, G. R., Parker, P. L. and Teal, J. *Analytical techniques for selected organic compounds.* In Goldberg, E. D., Ed., *Marine Pollution Monitoring: Strategies in a National Program,* Report of a NOAA-sponsored workshop at Santa Catalina Marine Biological Laboratory, October 1972.
5. Klimisch, H. J. and Reese, D. *J. Chromatogr. 80* (1973) 266.
6. Stedman, R. L., Miller, R. L., Lakritz, L. and Chamberlain, W. J. *Chem. Ind. (London)* (1968) 394.

Received March 2, 1979.