

Pyrolysis of asphaltenes and biomarkers for the fingerprinting of the *Amoco Cadiz* oil spill after 23 years

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The chemical composition of the petroleum products accidentally or deliberately released in the environment varies considerably with time under the action of biological (biodegradation) and physico-chemical (photo-oxidation) processes. It becomes more and more difficult to trace the origin of the oil spilled. A technique widely used for monitoring ancient oil pollutions is the study of oil biomarkers like terpanes and steranes^{1,2}. Here we show that the geochemical technique of asphaltenes pyrolysis can be successfully applied to environmental samples. This method allows the reconstitution of the original oil from the asphaltenes fraction of severely degraded oil residues. We applied the two techniques : biomarkers analysis and pyrolysis of asphaltenes to the long-term characterisation of the *Amoco Cadiz* oil 23 years after the spill in the salt marshes of Ile Grande, Northern Brittany, France. The results show that the oil reached the ultimate degradation stage. The total biodegradation rate was 60% relatively to initial oil. The asphaltenes pyrolysis generated a gas-chromatographic profile very similar to the original *Amoco Cadiz* oil. In the biomarkers fraction, gas chromatographic/mass spectrometric (GC/MS) analyses demonstrated that terpanes were conserved whereas steranes were partly degraded. We also showed that the class of seco-hopanes biomarkers are conserved and can be used in the long term monitoring of oil pollutions .

In the studies of environmental oil contaminations, the simple analytical techniques like conventional gas-chromatography with flame ionisation detection (GC-FID) reach rapidly their limits, due to the biological and physico-chemical weathering of oils³. In the course of the process, most compounds initially identifiable by GC-FID vanish, in the saturated like in the aromatic fractions of oil⁴. GC-MS techniques then become very useful, as they can trace degradation-resistant compounds. This is the case of the biomarkers terpanes and steranes which were widely used in geochemical as well as in environmental studies. Another geochemical technique is the pyrolysis of asphaltenes that is used in oil-source rock and oil-

oil correlations, and helps follow the secondary evolution process of reservoir oils⁵⁻⁷.

Asphaltenes are considered to be remnants of the fossil kerogen. The pyrolysis of asphaltenes under carefully controlled conditions mimic the process of generation of oil from the kerogen that occurred in geologic times and hence regenerate hydrocarbons similar to the original oil.

In the present work, we used biomarker analysis and, for the first time in an environmental study, the pyrolysis of asphaltenes to characterise the residues of the *Amoco Cadiz* oil stranded in sediments of northern Brittany coasts 23 years after the wreck. The accident occurred on 16 March 1978, and the 223 000 tons of Arabian light crude oil polluted about 360 km of shoreline. Numerous studies were carried out after the grounding⁸. Conversely, few researches have been conducted over a long period of time after an oil spill³.

Samples were collected in March 2001 in the salt marshes of the Ile Grande, where *Amoco Cadiz* oil was shown to persist by places⁹. The sediments were kept frozen (-20°C) until analyses. To evaluate the total weathering of the oil in these sediments, the chemical composition of the residual oil was compared to the original *Amoco Cadiz* oil that had been collected a few days after the wreck and kept frozen from this time.

Total residual hydrocarbons (HC) were extracted from defrost sediments by Soxhlet extraction with dichloromethane (DCM) during 8 hours. Their concentration was 3.14% wet weight. After solvent evaporation, the hydrocarbons of the initial oil (AMC) and those extracted from the sediments (IG) were separated with a SARA protocol into saturated, aromatic, resin and asphaltene fractions^{4,10}. Briefly, asphaltenes were precipitated in hexane and retained on glass microfiber filters. The filtrate (maltenes) was separated in saturates, aromatics and resins by liquid-solid chromatography. After evaporation of the solvents, each fraction was weighed. The saturated and aromatic fractions of AMC and IG were submitted to GC-FID analyses¹⁰. The saturated fractions were also analysed by GC-MS in the SIM mode to detect and quantify the biomarkers terpanes (m/z 191), steranes (m/z 217) and, for the first time in an environmental study, the seco-hopanes (m/z 123). The asphaltenes of IG were washed and pyrolysed (see detailed method below). The saturated fraction of the pyrolysate was analysed by GC-FID.

The figure 1 a shows the GC-FID chromatogram of the saturated fraction of the original *Amoco Cadiz* oil. The typical distribution of n-alkanes from nC10 to C40 was observed, together with the isoprenoids farnesane, nor-pristane (1650), pristane and phytane, and was characteristic of an Arabian light crude⁸. The lightest compounds below C13 had been lost by evaporation in the environment since the oil was collected several days after the wreck. The unresolved complex mixture (UCM) contains alicyclic saturated hydrocarbons.

The figure 1b shows the composition of the residual saturated HC in the Ile Grande sediments after 23 years of weathering. Almost all GC-resolvable HC were eliminated, which indicates that biodegradation was achieved, yet not total since part of the UCM still remains. These recalcitrant compounds constitute a stable form of organic matter and will never be totally removed from the environment⁴. Also visible on the chromatogram are the biomarkers steranes and hopanes that were further studied by GC-MS (see Fig.2). The most salient point is the chromatogram of the figure 1c that shows the distribution of HC released by pyrolysis of the asphaltenes of IG. When virtually no compound can be distinguished in figure 1B, all saturated HC from the initial AMC oil were regenerated by the pyrolysis, giving a fingerprint nearly identical to the original oil of figure 1a. Even light compounds below C8 were generated. The characteristic ratios C17/pritane and C18/phytane were similar in the initial oil (2.46 and 1.80) and in the pyrolysate (2.37 and 1.87). This indicates that pyrolysis of asphaltenes can be used most successfully in environmental studies of ancient oil pollutions. The GC-FID analysis of the aromatic fraction of IG showed that almost all GC-resolvable compounds were removed with the exception of some monoaromatic steroids and benzohopanes (data not shown).

Concerning the saturated heavy biomarkers, the figure 2a shows that the terpanes were not affected by long-term weathering, since their distribution is nearly identical in AMC and IG. This confirms the validity of using these compounds as conserved markers in long-term oil pollution monitoring¹¹. A less cited fact in oil pollution tracking is that steranes were not as stable as currently expected. It can be seen in figure 2b that C27 steranes were highly altered as well as the $\alpha\alpha\alpha$ 20R isomers of C28 and C29 steranes. As a result, these compounds should be used with caution in long term oil pollution studies. In the geological field, steranes were already known to be less resistant to biodegradation than terpanes¹². The class of seco-hopanes (figure 2c) is here tracked for the first time in an environmental study. These compounds^{13,14} appear here to be very stable and could be useful in researches on the topic.

According to the SARA protocol, the initial composition of the *Amoco Cadiz* oil was : 37.3% saturates, 34.7% aromatics, 15% resins and 13% asphaltenes. The composition of the Ile Grande residual oil was : 6.8% saturates, 14.6 % aromatics, 29.5% resins and 49% asphaltenes. If it is considered that resins are globally resistant to biodegradation^{4,10}, they can be used as stable internal standard. Consequently, the biodegradation rates of the different fractions of *Amoco Cadiz* oil after 23 years were 93% for saturates, 82% for aromatics, 0% for resins when an increase of 50% of asphaltenes was observed. This is highly compatible with previous data on the biodegradation of oil components⁴ that gave very similar results. The

persistant compounds are heavy polycyclic saturates and aromatics, and the totality of the resins. The asphaltenes concentration increased as a result of the production of stable metabolites resulting from the oxidation of degradable HC that are pooled with the true asphaltenes in the precipitation procedure. Overall, the total biodegradation rate of the *Amoco Cadiz* oil in the Ile Grande sediments was 60% after 23 years. It must be regarded as the highest degradation rate that can be attained, thus showing that crude oil degradation is never complete and that some oil compounds and stable metabolites can persist on the very long term in the environment. The potential ecological impact of these persistant compounds is not yet known.

Methods

Pyrolysis of asphaltenes.

The asphaltenes of the total residual oil extracted from IG sediments were precipitated at room temperature in hexane during a time contact of 12 h. The hexane containing insoluble asphaltenes and soluble maltenes was filtered on Whatman GF/F glass microfiber filters that retained the asphaltene fraction. The filters were then washed with boiling hexane in Soxhlet apparatus for 8 hours. Preliminary trials showed that this purification step is essential to remove metabolic byproducts resulting from HC biodegradation like fatty acids, esters and ketones that co-precipitate with true asphaltenes and generate artifacts during pyrolysis. The washed asphaltenes were Soxhlet extracted from the filters for 2 hours with DCM and about 20mg were transferred dried in glass tubes (100x 6 mm in diameter) for closed mild pyrolysis^{15,16}. The tubes were sealed with a flame under nitrogen then placed in a gas chromatograph oven at 320°C for 48 hours. The temperature and time of the pyrolysis were fixed after a series of trials conducted with a view to obtain the most similar chromatograms between original AMC oil and pyrolysate. After the heating period, the tubes were cooled at room temperature, opened and the pyrolysis products were recovered with hexane by ultrasonication (40kHz, 20 mn). The hexane soluble pyrolysate was passed on a 100-200 mesh activated silicagel microcolumn so that only the saturated fraction of the pyrolysate was collected. This saturated pyrolysate fraction was analysed by GC-FID.

1. Wang, Z.D. & Fingas, M. Developments in the analysis of petroleum hydrocarbons in oils, petroleum products and oil-spill-related environmental samples by gas chromatography. *J. Chromatogr. A* **774**, 51-78 (1997).

2. Wang, Z.D., Stout, S.A. & Fingas, M. Forensic fingerprinting of biomarkers for oil spill characterization and source identification. *Environ. Forensics* **7**, 105-146 (2006).
3. Prince, R.C. *et al.* The roles of photooxidation and biodegradation in long-term weathering of crude and heavy fuel oils. *Spill Science & Technology Bulletin* **8**, 145-156 (2003).
4. Oudot, J. Rates of microbial degradation of petroleum components as determined by computerized capillary gas chromatography and computerized mass spectrometry. *Mar. Environ. Res.* **13**, 277-302 (1984).
5. Rubinstein, I., Spyckerelle, C. & Strausz, O.P. Pyrolysis of asphaltenes : a source of geochemical information. *Geochim. Cosmochim. Ac.* **43**, 1-6 (1979).
6. Petrov, A.A. Petroleum hydrocarbons. Spriger-Verlag, Berlin (1987).
7. Magnier, C. & Huc, A.Y. Pyrolysis of asphaltenes as a tool for reservoir geochemistry. *Org. Geochem.* **23**, 963-967 (1995).
8. CNEXO ed. *Amoco Cadiz*, fates and effects of the oil spill. Paris (1981).
9. Mille, G., Munoz, D., Jacquot, F., Rivet, L. & Bertrand, J.C. The *Amoco Cadiz* oil spill : Evolution of petroleum hydrocarbons in the Ile Grande salt marshes (Brittany) after a 13-years period. *Est. Coast. Shelf Sci.* **47**, 547-559 (1998).
10. Chaillan, F. *et al.* Identification and biodegradation potential of tropical aerobic hydrocarbon-degrading microorganisms. *Res. Microbiol.* **155**, 587-595 (2004).
11. Prince, R.C. *et al.* 17 α (H),21 β (H)-hopane as a conserved internal marker for estimating the biodegradation of crude oil. *Environ. Sci. Technol.* **28**, 142-145 (1994).
12. Moldowan, J.M. *et al.* Source correlation and maturity assessment of select oils and rocks from the central Adriatic Basin (Italy and Yugoslavia) : In Moldowan, J.M., Albrecht, P.& Philps, R.P. (Eds), Biological markers in sediments and petroleum. Prentice Hall, Englewood Cliffs, New Jersey, 370-401 (1992).
13. Schmitter, J.M., Sucrow, W. & Arpino, P.J. Occurrence of novel tetracyclic geochemical markers: 8,14-seco-hopanes in a Nigerian crude oil. *Geochim. Cosmochim. Ac.* **46**, 2345-2350 (1982).
14. Fazeelat, T., Alexander, R. & Kagi, R.I. Molecular structures of sedimentary 8,14-secohopanes inferred from their gas chromatographic retention behaviour. *Org. Geochem.* **23**, 641-646 (1995).
15. Monthioux, M., Landais, P. & Durand, B. Comparison between extracts from natural and artificial maturation series of Mahakam delta coals. *Org. Geochem.* **10**, 299-311 (1986).

16. Cassani, F. & Eglington, G. Organic geochemistry of Venezuelan extra-heavy oils. *Chem. Geol.* **56**, 167-183 (1986)
17. Peters, K.E., Walters, C.C. & Moldowan, J.M. The biomarkers guide, second ed. Biomarkers and isotopes in petroleum systems and Earth history, vol. II. Cambridge University Press, UK (2005).

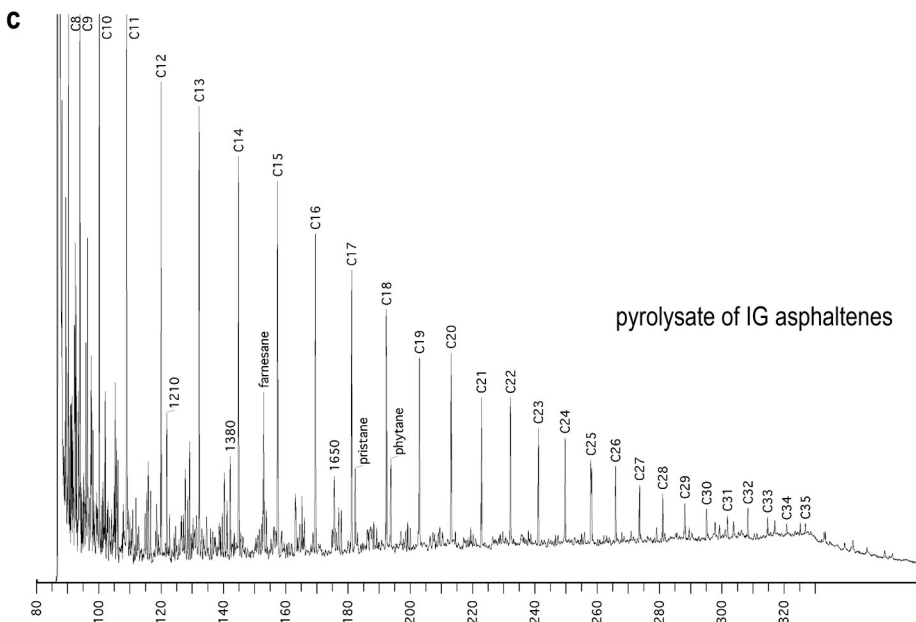
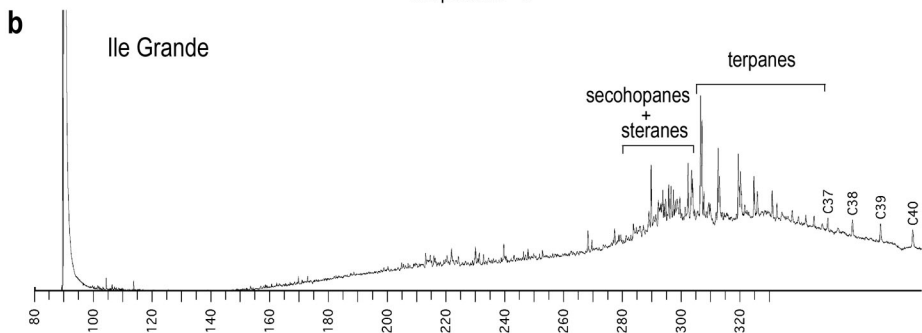
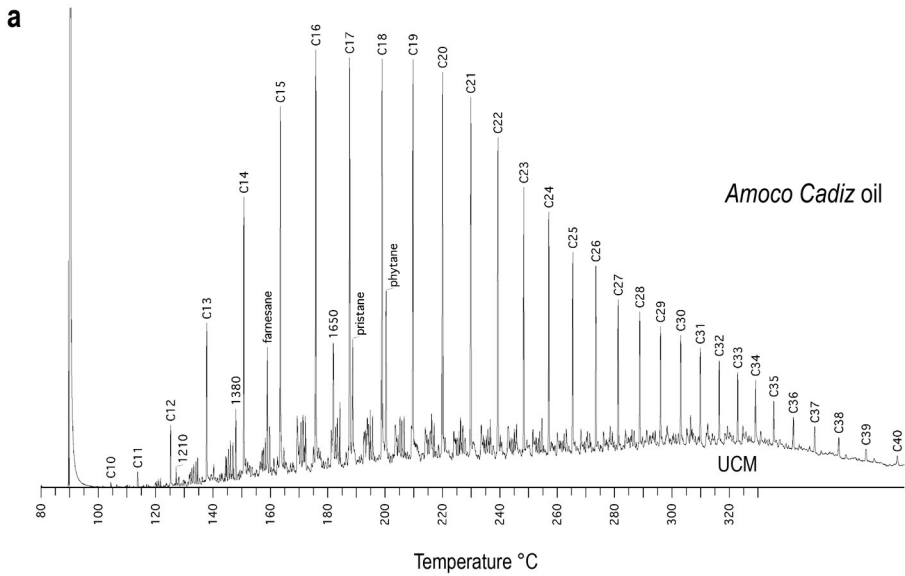
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Figures legends

Figure 1. GC-FID analyses of the saturated fractions. **a**, initial *Amoco Cadiz* oil. **b**, HC extracted from the Ile Grande sediments after 23 years. **c**, pyrolysate of the Ile Grande asphaltenes. Ci refer to the number of carbon atoms in the n-alkane chain, intermediate peaks are iso- and branched alkanes. UCM, unresolved complex mixture.

Figure 2. GC-MS analyses of the biomarkers¹⁷ (abbreviated nomenclature). **a**, terpanes. 1, Ts; 2, Tm; 3, C29 $\alpha\beta$ norhopane; 4, C30 $\alpha\beta$ hopane; 5 and 6, C31 $\alpha\beta$, 22S and 22R; 7 and 8, C32 $\alpha\beta$, 22S and 22R; 9 and 10, C33 $\alpha\beta$, 22S and 22R; 11 and 12, C34 $\alpha\beta$, 22S and 22R; 13 and 14, C35 $\alpha\beta$, 22S and 22R. **b**, steranes. 1, $\alpha\alpha\alpha$ 20S; 2, $\alpha\beta\beta$ 20R; 3, $\alpha\beta\beta$ 20S; 4, $\alpha\alpha\alpha$ 20R. **c**, secohopanes. 1, C29 8-14 secohopane; 2, C30 8-14 secohopane; 3, C31 8-14 secohopane.



Amoco Cadiz oil

Ile Grande

