

**MUD GAS LOGGING IN EXPLORATION WELLS.
DO WE NEED A MASS SPECTROMETRIC DETECTION OR ONLY A
TRADITIONAL GC/FID MUD GAS LOGGING?**

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Mud gas logging is employed in most exploration wells as a screening tool for gas composition, hydrocarbon yields and safety aspects. The method is based on GC/FID detection (Gas Chromatography / Flame Ionization Detection) of typically C₁-C₅ hydrocarbons and CO₂. The compound separation and analytical selectivity is limited and restricted by the column resolution, selected cycle time and measurement density.

The quality of a GC/FID method depends on the resolution power of the separating column. A MS detector (Mass Spectrometer) will enhance the compound selectivity and thereby, report more accurate results. The technique is based on a sophisticated instrumentation, which may be more prone to malfunctions than a simple GC system.

The GC method is based on compound separation by using a capillary column. The compound specific fragmentation pattern by the mass spectrometry technique is unique and may expel the necessity of a GC separation. A stand-alone MS-technique benefits from a reduced cycle time (more rapid measurements) and increased carbon number detection range. An MS system is generally more sensitive than a FID system, in showing higher signal to noise ratio.

This study evaluates two different commercially available mud gas MS systems applied in two different exploration wells. The geochemical interpretations of the well profiles are provided and the MS datasets are compared methodwise as well as to the standard GC/FID mud gas data.

The GCMS instrumental setup was employed in a well comprising an almost 300 m thick interval of oil rich sediments whereas the stand-alone MS instrumental setup was employed in a well in part comprising gas-condensate bearing sediments. It will be shown that both techniques are feasible for characterizing the hydrocarbon type present while drilling, i.e. gas, condensate or oil.

In the case of wells containing rather dry gas, the MS also provides analyses of several inorganic compounds as helium, CO₂ and sulphur compounds. These may add important information in interpreting any potential differences in the gas bearing strata.

However, when secondary alteration effects in hydrocarbon mixtures were encountered in some zones (biodegradation and waterwashing) it was an advantage to run the GCMS instrument since both the iso and normal hydrocarbon compounds were more adequately analyzed in the C7(-) range and hence provided a better basis for more detailed follow-up sampling and analysis. In practice this advantage has to be weighed against the total costs, which were several times higher for the GCMS setup compared to the stand-alone MS setup.

A RAPID SCREENING METHOD FOR ESTIMATING SOURCE ROCK MATURITY FROM CORE AND CUTTING SAMPLES

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A rapid and cost-effective method for estimating the maturity of source rocks using fluorescence spectrophotometry has been developed. The method involving the direct analysis of hydrocarbon extracts from source rocks using the Total Scanning Fluorescence (TSF; Barwise and Hay 1996) technique. Prior to the solvent extraction core and cutting samples were cleaned using a standardised procedure with a water based weak oxidising agent to ensure the removal of contaminants while retaining the indigenous hydrocarbons sealed in the micro-pores.

Investigation of a number of wells from two basins in China and Australia on both Type I and II source rocks indicates that the method can be used to effectively estimate the maturity levels of source rocks, which are comparable to that obtained using vitinite reflectance (V_r) and Rock Eval data (Figure 1) with a correlation coefficient (R^2) between V_r and TSF maturity parameter, R_1 , greater than 0.90 for V_r values of >0.55 .

As exemplified by the Paqualin-1 well (Figure 1), the oil generation window is characterised by high (maximum) and constant TSF intensity and increasing of TSF maturity indicator (reducing R_1 values) with depth; whereas the onset of the gas generation window is marked by an abrupt drop of the TSF intensity and continued increasing of TSF maturity indicator with depth and a characteristic condensate TSF spectrogram.

Compared with conventional maturity indicators such as Vitrinite Reflectance (V_r) and Rock Eval data the new method is relatively rapid and cost-effective, and can detect the onset of immature hydrocarbons and maturity variations within the gas window (Figure 1).

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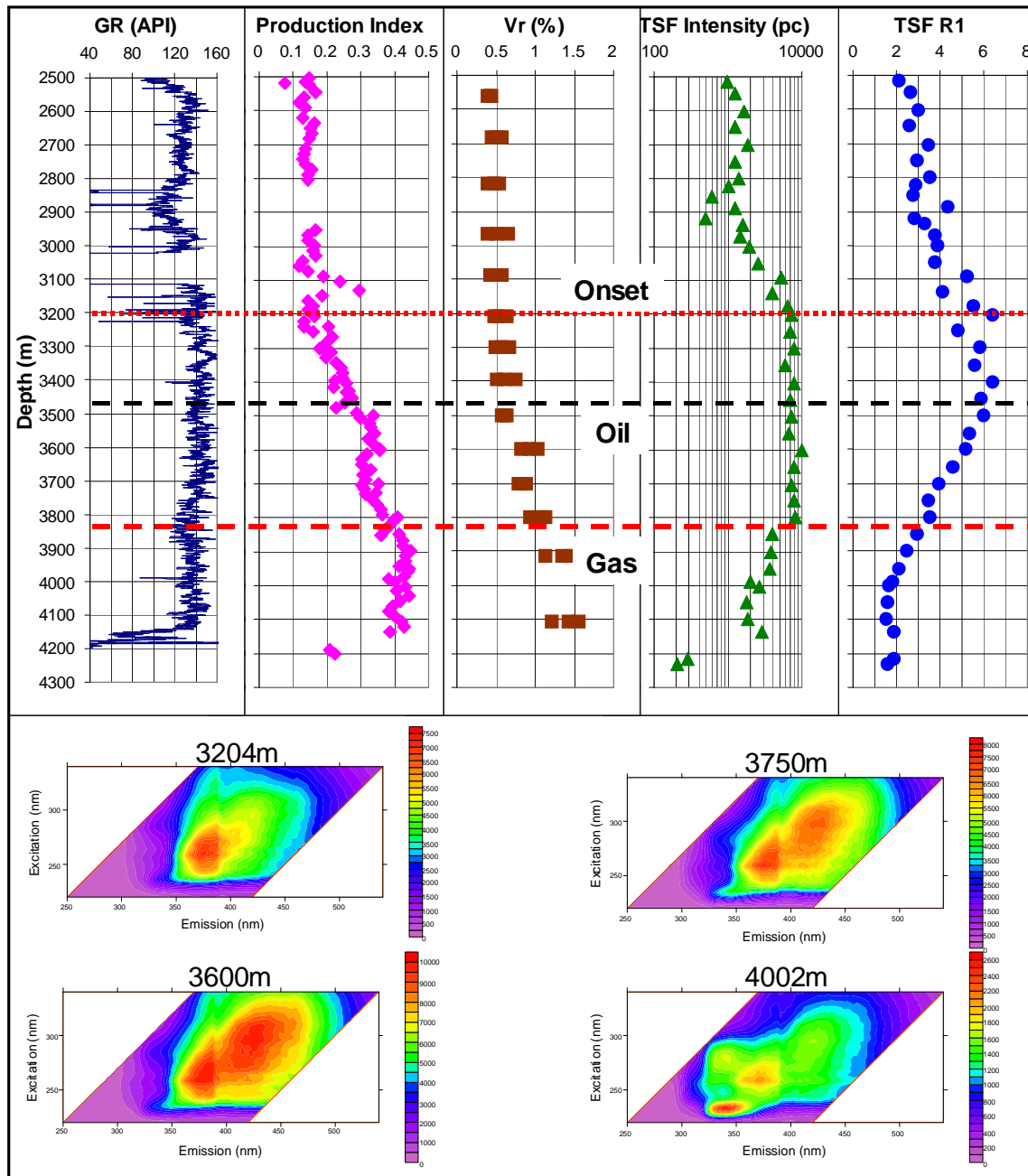


Figure 1. Selected TSF spectrograms and depth profiles of TSF Intensities, TSF R1 parameter of Paqualin-1 well, Vulcan Sub-basin, Timor Sea, Australia in comparison with depth profiles of Production Index (Rock Eval data) and Vitrinite Reflectance values. The inferred depths of the onset of immature hydrocarbon generation and the modelled depths of the oil and gas windows are indicated.

ESTIMATION OF API GRAVITY BY QUANTITATIVE GAS CHROMATOGRAPHY FOR SELECTED OILS OF BRAZILIAN BASINS

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API gravities strongly impact the economic viability of oil fields because physical properties of oil affect oil value and its production (e.g. Tissot and Welte, 1984; Peters & Moldowan, 1993). Furthermore, even after decision has been made to develop a field, API gravity continues to impact the production strategy, since variations of this property must be considered for optimal well location and completion. However, API gravity generally cannot be measured by conventional methods before production tests because of the small amount of oil extracted from cuttings and core samples during drilling. Quantitative gas chromatography, a technique that is used to analyze volatile organic compounds, can be proposed as a technique to quickly estimate the API gravity of oils with acceptable precision. The main advantage of its use is that the amount of sample required is minimal. It can be fast and bring many benefits to a quick evaluation during well drilling and testing when the amounts of crude oil available are small. In addition, gas chromatography accounts for the large range of compounds and molecular weights present in oils.

The aim of this study was to develop a gas chromatographic method to quantify petroleum fractions ranging from 7 to 40+ carbon atoms in Brazilian oils and to estimate oil API gravities, using two newly developed macros compatible with the Agilent Chemstation.

GC analyses were performed with a Hewlett-Packard 6890 series II gas chromatograph equipped with an on-column injector and a flame ionization detector (FID). A retention gap and fused silica DB-5 phenyl/methyl polysiloxane capillary column (20 m x 0.25 mm x 0.1 μ m) were used. The oven temperature was programmed from 35°C to 340°C at a heating rate of 2.5°C/min. Helium was used as carrier gas at a constant flow of 2.8 mL/min. Around 10mg of sample were diluted in 1mL of carbon disulphide. A standard mixture containing *n*-paraffins from 5 to 28 carbon atoms (*Analytical Controls, part number 59.50.101A*) dissolved in CS₂ was used to calibrate retention times and to calculate response factors.

Two macros have been developed for quantification of the GC traces. The first one runs on a standard containing some pure *n*-alkanes from *n*-C₅ to *n*-C₂₈. This macro calculates the response factor that is applied to all whole-oil samples. The second macro gives

cumulative mass percentage results of slices of the chromatogram delimited by the peaks of *n*-paraffins (including hump) observed in the oil. A final reported value of total cumulative mass is obtained, and the difference between this value and the injected mass is attributed to the amount of sample retained inside the chromatographic column, which corresponds to the non-eluted compounds representing sample loss. Furthermore, quantitative GC with the macro provides the mass fraction of the pre-selected peaks (*n*-paraffins, pristane, phytane) as well as the mass fraction of the sum of all resolved peaks and the hump (UCM) area. Oil density is estimated by calculating a weighted average, i.e., the summation of the products of mass fraction and density values for each slice of the chromatogram:

$$\sum_{i=1}^n \rho_i \times w_i, \text{ where } \rho_i \text{ and } w_i \text{ are the density and mass fraction respectively of each "i" slice.}$$

API gravities can then be calculated from the estimated whole-oil density.

To ascertain that the analytical conditions and the macro that calculates the response factor provide correct results, six samples of the standard mixture containing *n*-paraffins from *n*-C₅ to *n*-C₂₈ were analyzed. Mean, standard deviation, % standard deviation for the six response factors were calculated and the results were considered adequate since the % standard deviation was less than 3%. These tests attest the repeatability of the results and the correct data processing of the developed macro.

The optimized method was applied to a set of Brazilian oils with a wide range of API gravities (8 to 45°), ranging from non-biodegraded to intensely biodegraded oils. Our results showed an excellent correlation between estimated and measured API gravities for each oil family that was investigated. To achieve this, specific exponential functions defining the relationship between pseudo-densities and slices along the GC trace have been numerically fitted for each oil family. The results of this study corroborated the usefulness of applying quantitative gas chromatography in the geochemical assessment of oils including their physical and chemical properties.

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AN IMPROVED TITRATION METHOD FOR DETERMINATION OF ACID AND PHENOL CONTENT IN CRUDE OILS COMPARED TO EXTRACTION AND HPLC CHARACTERISATION

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The standard method for determining the total amount of acidic compounds in crude oils is by non-aqueous titration with potassium hydroxide, and gives a total acid number – TAN – which is intended to be a measure of all acidic functions in the sample (ASTM D-644-81, 1987). This procedure accurately determines the concentration of carboxylic acids and compounds of equivalent acidity in the sample. However, phenolic compounds and other weaker acids are not within the range of acidities covered by the method. The TAN determination thus gives no information about a considerable part of the compounds that in other contexts are included in the acid fraction, and which may be important for many physical properties of the oils, e.g. surface activity in mineral-oil-water interactions, emulsion stability and corrosion risk.

The range of compounds determined by non-aqueous titration is a function of the solvent system and strength of the base. Using an alternative solvent system, we have developed a procedure that determines the concentration of compounds both in the carboxylic acid pK_a range (TAN-C) and in the phenolic pK_a range (TAN-P) in crude oils in a single titration. The results show that the concentration of the titrable phenolic compounds is comparable to the stronger acid content for biodegraded oils with high TAN values, and significantly higher in low TAN oils, see Figure 1.

Data presenting the scope of the improved TAN determination will be presented, and compared to the results from ion exchange based extraction of acids and HPLC determination of the acid composition using a recently developed procedure that separates the acids into fractions based on acid strength (Borgund *et al.*, 2007). The differences in distribution of the acids in biodegraded and non-biodegraded oils will be focussed.

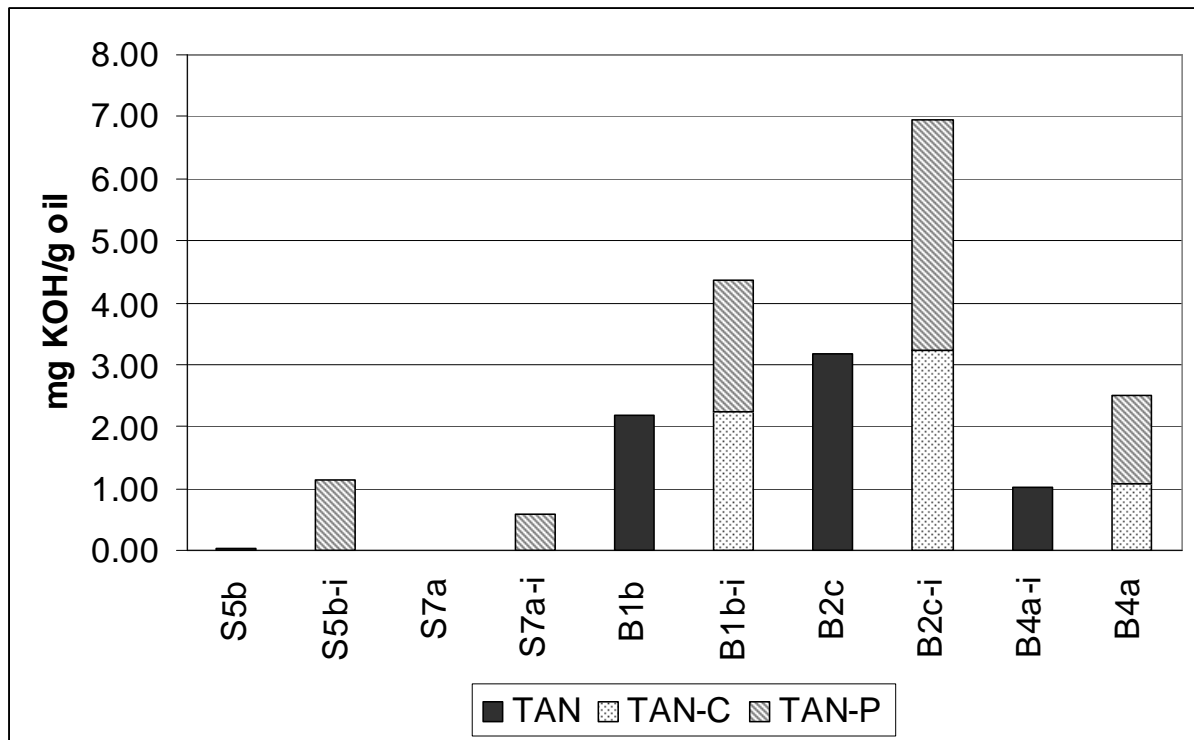


Figure 1. TAN by conventional titration compared to TAN-C and TAN-P values for 5 North Sea Crude oils. Oils S5 and S7 are non-biodegraded and oils B1, B2 and B4 are biodegraded. The results labelled “i” are from the improved titration method.

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RAPID AND EFFICIENT PROTOCOL FOR THE SEMI-QUANTITATIVE ANALYSIS OF C₂₊ CARBOXYLIC ACIDS IN CRUDE OILS

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A wide range of carboxylic acids has been detected in minor to moderate amounts in many oils. Detected species include alkanolic acids (Rodrigues et al., 2000), naphthenic acids (Aitken et al., 2004) and hopanoic acids (Pan et al., 2006). These compounds are not routinely analysed due to laborious workup procedures, often with low or inaccurate recoveries. The methods often require large amounts of solvent which prevents the analysis of low molecular weight carboxylic acids due to evaporative losses during sample preparation.

Here we present an alternative method for the analysis of carboxylic acids from crude oils. The protocol is based on small-scale column (Pasteur-pipette) liquid chromatography and allows the rapid throughput of large sample sets. After eluting hydrocarbons and heterocycles with apolar and aromatic solvents, a polar acidic fraction is recovered with diethyl ether (2% formic acid). Following the butylation of this fraction, non-carboxyl interferences are removed on a silica column.

Recoveries are substantially higher than previously published methods (up to 100%) and are reproducible and consistent, thus allowing for accurate quantification. To prevent the loss of low molecular weight carboxylic acids, the method uses a minimum amount of solvent and the recovered acids are derivatised to *n*-butyl esters. In this manner compounds as volatile as acetic acid (C_{2:0}) can be quantitatively measured when using corresponding deuterated surrogate standards. In addition, multifunctional acids and long-chain alkanolic acids are also semi-quantitatively recovered in the same fraction.

Test trials revealed the optimum conditions for the Fischer esterification reaction (time, temperature, reagents) using sulphuric acid (H₂SO₄) and boron trifluoride (BF₃) as catalysts. The use of H₂SO₄ has a number of advantages over BF₃, since the latter reagent has a limited shelf-life, forms a health hazard and can lead to artefacts during the workup procedure.

We will present a clear depiction of the described method in an attempt to encourage the analysis of carboxylic acids which should lead to an increased understanding of the origin and fate of this compound class in petroleum.

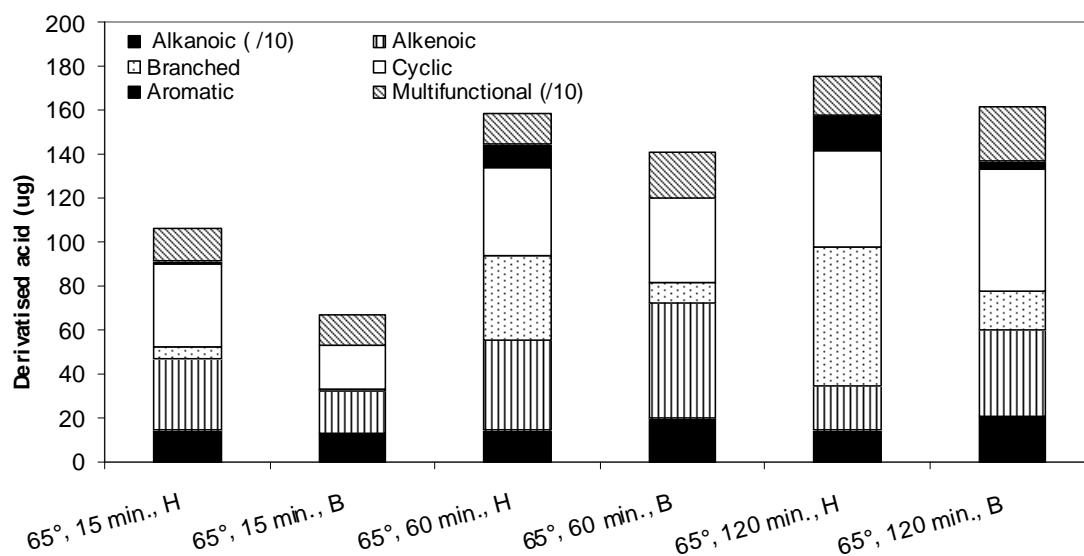


Figure 1. Compared butylation efficiency using 100ul n-butanol. Time and temperature conditions, respectively catalyst (B- BF_3 ; H- H_2SO_4) are listed on the x-axis. Note the greater derivatisation efficiency when using H_2SO_4 , which is particularly pronounced in aromatic and branched acids.

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TLC-FID (IATROSCAN) ANALYSIS OF HEAVY OIL AND TAR SAND SAMPLES

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TLC-FID (thin layer chromatography-flame ionization detector, Iatroscan) has long been used as a rapid and inexpensive way to determine SARA fractions (saturate + aromatic = hydrocarbon, resin + asphaltene = polar) in crude oils and bitumen extracts. However, the analysis of heavy oils and tar sands (HOTS) poses a number of additional problems. HOTS have relatively high amounts of polar compounds that are retained near the origin on TLC rods, potentially causing both separation and quantitation problems. Additionally, the polar compounds have variable quantities of heteroatoms resulting in different FID response factors and thus further complicating the quantitation of the SARA fractions.

Two operational variables have been optimized with respect to improving the reproducibility of the Iatroscan data generated for HOTS samples. In addition to internal reproducibility, these operational parameters were also investigated with respect to obtaining SARA results that were more directly comparable to determination by asphaltene precipitation followed by open column chromatography using a silica gel alumina/column.

The first variable was to simply consider the size of the spot in which the sample was initially applied to the rod. Counterintuitively, it was observed that a very small spot (~2 mm) applied using an automated spotting device did not provide for better separation of the SARA fractions. More reproducible results were obtained by using a relatively rapid injection resulting in an initial spot size of about 5-6 mm. This result stems from the fact that SARA fractionation is not a normal chromatographic separation but rather a fractionation based on moving different polarity groups different distances up the rod by developing the rods with a series of different solvents to different distances. The apparent loss of resolution with the very small spot size may result from overloading of the silica at the origin and commensurate channeling of the low polarity solvents through the spot.

The second variable tested was to alter the properties of the silica rods by doping them with a small amount of transition metal. The effect of this was to increase the absolute response of the hydrocarbon fractions (Fig.1), especially the saturate compounds and to reduce the relative concentration of the polar compounds. Doping the rods with transition metal may also help to minimize the variability of response factor of the polar fractions.

Iatroscan SARA analysis of individual fractions obtained from asphaltene

precipitation and open column chromatography demonstrates that the two analytical techniques do not provide precisely the same separation. This is not surprising because the open column technique generally is carried out with both silica gel and alumina absorbents whereas the Iatroscan was carried out on silica rods or metal doped silica rods. It may not be possible to make a direct comparison of data generated by the two techniques because the polar fractions are fundamentally different and the Iatroscan technique also incorporates a response factor that is not an issue in the gravimetric detection of fractions from column chromatography. However, Iatroscan may well provide a higher degree of reproducibility of the amounts of polar fractions than column chromatography in the case of HOTS samples. In part, this reduced scatter may result from reduced oxidation during the TLC fractionation, reduction of physical occlusion, and also because evaporation of the solvent from a partially volatile sample is more consistent.

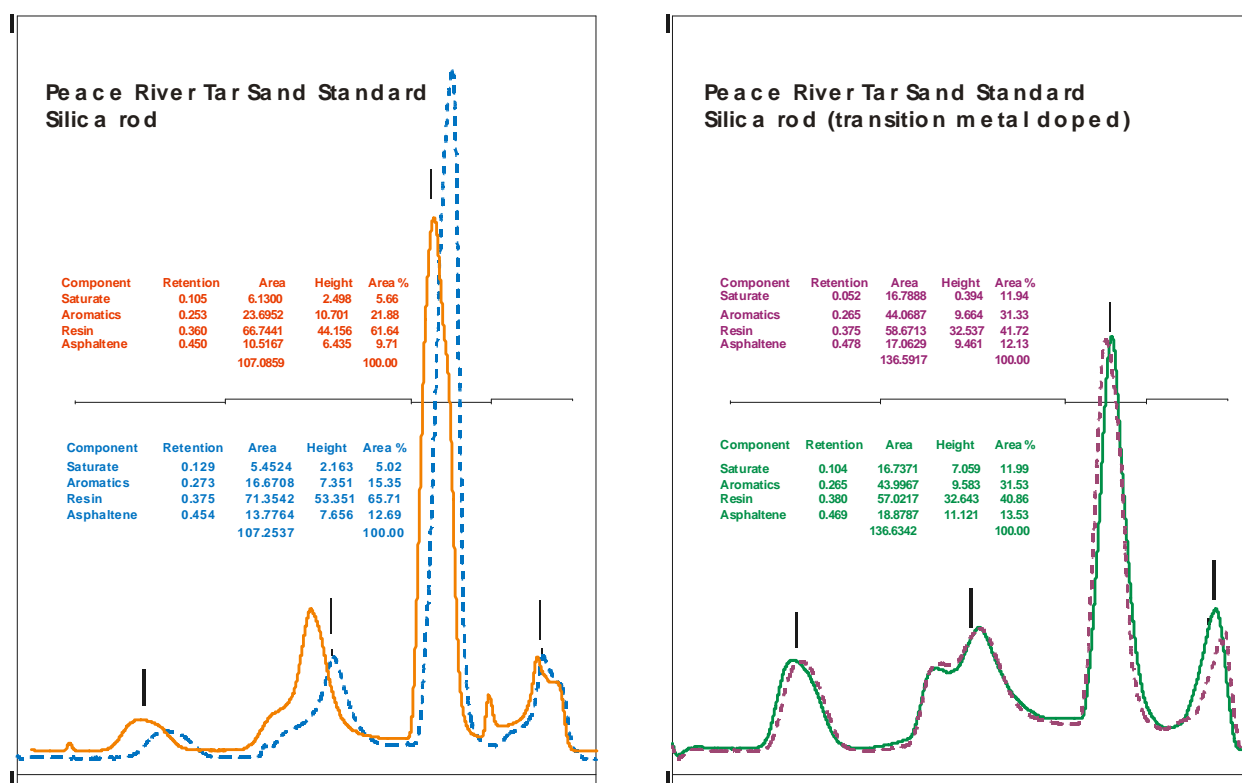


Figure 1. Comparison of absolute response and reproducibility of Iatroscan SARA analysis of a sample of Peace River Tar Sand bitumen.

APPLICATION OF GAS-CHROMATOGRAPHY USING THE CARBURANE SOFTWARE IN THE OIL CHARACTERIZATION FROM DIFFERENT RESERVOIRS

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Geochemical petroleum characterization allows the recognition of depositional paleoenvironment of source rocks, the level of maturation of the oil and possible secondary processes that can change the oil composition after the reservoir is filled.

The geochemical characteristics of reservoired fluids in a Brazilian oil field were studied by gas chromatography analysis (GC) with the CARBURANE software combined with statistical data analysis. CARBURANE is a chromatography data processing software that provides detailed characterization and quantification of hydrocarbons in petroleum (Ferreira *et al*, 2006). Originally developed for refining purposes, it allows the identification of the whole set of isomers of organic compounds with 5 to 15 carbon atoms. Currently, this software is being used in Petrobras for reservoir geochemistry and characterization studies, even for biodegraded oils with low API gravities.

The aim of this study was to apply this new analytical methodology (GC-CARBURANE) to characterize oils produced from an oil field located in the central part of Campos Basin, Brazil. There are two producing intervals that involve Oligocene (the shallowest ones) and Paleocene-Eocene reservoirs (the deepest ones). The produced oils have the same origin and maturation according to biomarker results, and migrated from the same source kitchen. Pressure and seismic reservoir data suggested the possible communication between the reservoirs through normal faults. Thus, this new quantitative methodology was used to identify compositional differences associated with variations in the biodegradation intensity.

The analytical results were applied to calculate ratios between linear paraffins and adjacent saturated compounds using their concentrations in mass percentage and these ratios bring out the small differences in biodegradation among the crude oils. The linear paraffins are more susceptible to the biodegradation process when compared to the adjacent compounds and are more appropriate for the evaluation of the early stages of biodegradation. The statistical analysis shows two different oil groupings and the resulting dendrogram is shown in

Figure 1. The first group comprises the produced oils from the shallowest reservoirs and the second group involved those from the deepest reservoirs, suggesting that the two production zones are not in communication anywhere in the oil field.

In conclusion, this approach allowed us to discriminate produced oils from adjacent reservoirs containing oils with similar molecular composition, besides can suggesting no oil zone communication among these reservoirs.

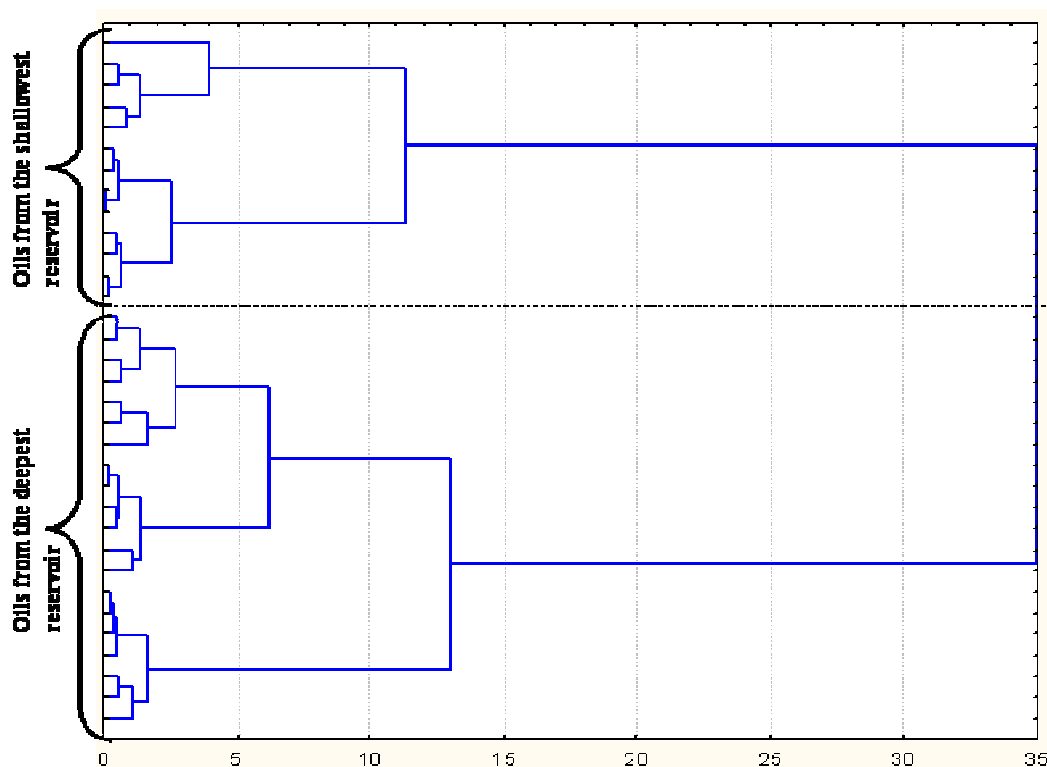


Figure 1. Dendrogram obtained from the cluster analysis of GC data processed with the CARBURANE software from a set of crude oils from a field in central part of Campos Basin, Brazil.

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COMPARATIVE STUDY OF ARTIFICIAL INTELLIGENCE TECHNIQUES IN OILS CLASSIFICATION FROM BRAZILIAN SEDIMENTARY BASINS EMPHASIZING DECISION TREES

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Nowadays geochemists have a large variety of geochemical data available to provide information about the paleoenvironmental characterization of oils (PETERS *et al.*, 2005). Since interpretation of this information is usually made through a visual analysis of many geochemical data, a technique that could provide the classification of these different paleoenvironments using the most important parameters would be very useful in the process of oil characterization.

The objective of this study was to evaluate the performance of several artificial intelligence techniques like decision trees, decision rules and neural networks in the classification of oils from different origins, trying to optimize the process of data interpretation and oil classification. The results of these classification techniques were compared with the classifications provided by previous geochemical studies.

The database for this classification study consisted in 2924 oil samples with different origins (lacustrine, marine and mixed), degrees of thermal evolution, and distinct levels of biodegradation from different Brazilian sedimentary basins. The parameters used as input variables in the classification model were the results of the following analyses: bulk, gas chromatography, liquid chromatography, stable carbon isotopes and GCMS and GCMSMS (biomarker ratios). The variables mostly affected by biodegradation and thermal alteration processes of oils had to be removed, to provide generic model using variables mostly related to the oils origin.

Firstly the database was submitted to a preliminary exploratory study to evaluate inconsistencies in data and *outliers* (samples with abnormal behavior) through boxplots and clusters and correlations between variables using scatterplots. After that several classification artificial intelligence techniques (QUINLAN, 1993), decision rules (WITTEN & FRANK, 1999), and neural networks (HAYKIN, 1999) and with special emphasis in decision trees were employed to evaluate the accuracy in classification of oils from different origins.

Using these techniques, classification results have shown that by using only 22 geochemical parameters it is possible to obtain a good match with a previous classification

given by the specialist geochemists with accuracy above 90%. For the subtypes where the number of samples is less than 10 the accuracy was reduced. The samples classification was made in two levels: first to classify in the 3 principal types of oils (lacustrine, marine and mixed) and later the classification in subtypes (e.g. lacustrine saline, lacustrine freshwater, marine siliciclastic, marine evaporitic, etc.).

If compared with the other classification techniques employed in this study, decision tree presented the best results, especially because this method provides the most important parameters selected to distinguish the different classes and the interval of occurrence of each parameter for each class, contrasting with neural networks that only shows the % accuracy in the classification. In the figure 1 the decision tree used for the classification of mixed oils is show, where it is possible to examine all the parameters selected for the discrimination between the groups and its values. The parameters are ratios between C_{26} and C_{28} tricyclic terpanes (26/28 TRI) and ratio between C_{30} tetracyclic polyprenoids (21R + 21S) and C_{27} diasteranes (20R + 20S) (TPP).

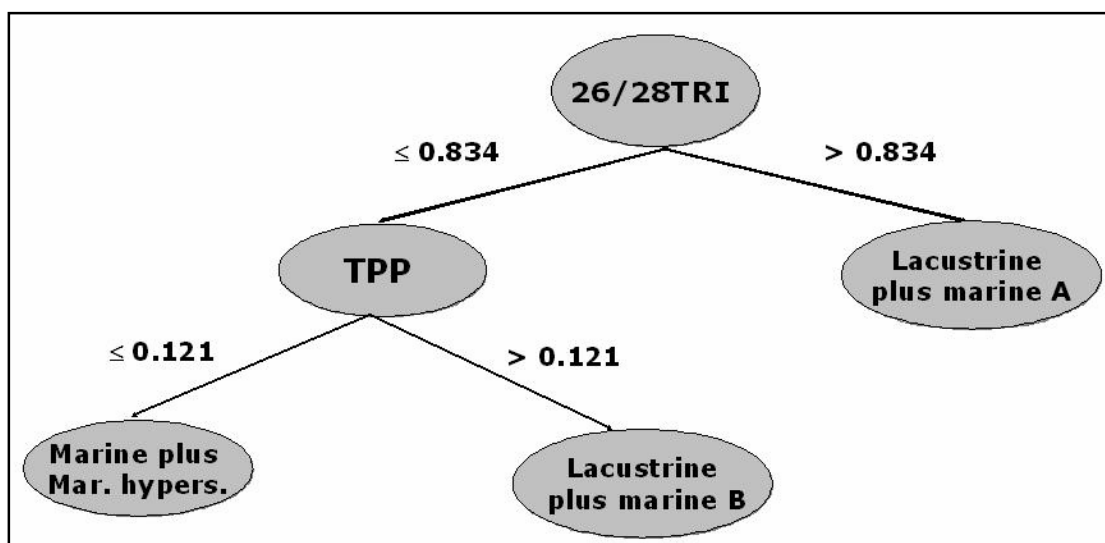


Figure 1. Decision tree obtained for the classification of mixed oils, showing the biomarker ratios used for the classification in the Lacustrine plus marine A, Lacustrine plus marine B and Marine plus marine hypersaline.

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FLUORESCENCE LIFETIME ANALYSIS OF SINGLE HYDROCARBON-BEARING FLUID INCLUSIONS – A PARAGENETIC PERSPECTIVE

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UV fluorescence is commonly used as a first-pass visualisation tool for the study of Hydrocarbon bearing Fluid Inclusions (HCFI). Fluorescence colour is commonly used as a guide to estimate API gravity (Bodnar, 1990) and allow visual discrimination of single or multiple HCFI generations into a paragenetic context. Bulk HCFI crushing with GC analysis has also been compared in an effort to link fluorescent colour and petroleum chemistry (George et al, 2001). However, the human eye is easily deceived by colours and thus observing fluorescent colours is at best a semi-qualitative technique. In contrast, fluorescence lifetimes provide quantitative measurements that are: repeatable, unaffected by host mineral colour, inclusion size, or inclusion dimensions. Fluorescence lifetime measurements correlate with crude oil composition (Ryder, 2004) and lifetime measurements on HCFI have previously been measured by single-photon counting technology (Ryder et al., 2004).

In this work we present new data based on frequency-domain (FD) lifetime measurements and full Fluorescence Lifetime Imaging Microscopy (FLIM) imagery. The FD method uses a modulated (1-300 MHz) 405 nm laser excitation source and calculates the fluorescence lifetime by measuring the phase delay and demodulation ratio of the fluorescence signal relative to the modulated excitation source. The FLIM system we use has an optical resolution of <1 micron, a motorised XYZ stage, and is capable of generating 3D FLIM images of HCFI in the low micron size range. The fluorescence lifetime data is generated via a variety of data fitting models which can be correlated to crude oil composition. Using single modulation frequency measurements we are able to produce FLIM images rapidly that can discriminate different HCFI generations very clearly. Using the more time consuming multi-frequency single-point average lifetime measurements we get very accurate and reproducible lifetime data that can be used to estimate oil composition. The variation in average lifetime of HCFI or crude oils is attributed to the interplay between energy transfer and quenching, which is a function of the oil composition.

To date we have collected data from a diverse selection of HCFI's and measured lifetimes in 0.6 to 25 ns range, with most measurements being between 1.8 and 10 ns with a precision that is better than 5%. Measurements also confirm that lifetimes are homogeneous

within individual HCFI and are independent of fluorescence intensity. HCFI's that fluoresce red or orange during UV excitation generally have the shortest lifetime measurements whereas the longest lifetimes are associated with HCFI's that fluoresce blue in colour (by visual observation). For groups of HCFI that have the same apparent fluorescence colour, we are also able to discriminate HCFI groups on the basis of lifetime. The opposite is also true, and for one sample of a single HCFI generation, the large inclusions fluoresced white whereas very thin inclusions had an apparent blue fluorescence, but lifetime measurements gave the same average lifetime indicating that the composition is the same.

Petroleum-related samples are commonly host to multiple HCFI generations. The average lifetimes for an individual generation are the same whereas other generations invariably have different average lifetime measurements. By establishing the paragenetic relationships between each HCFI generation from careful studies under plain and UV fluorescent light we have documented that lifetime measurements generally increase with each successive inclusion generation. An exception to this rule resulted from a secondary petroleum fluid being captured as an additional HCFI generation.

In conclusion, FLIM on HCFI's provide repeatable measurements and are unaffected by signal intensity, inclusion size or shape, or host mineral colour. Fluorescent lifetimes are quantitative, allowing us to both discriminate HCFI populations and improve paragenetic studies. Unlike the human eye which is deceived by fluorescent colour and intensity, FLIM is definitive, providing a valuable tool to enhance the study of HCFI.

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A NEW REACTOR FOR OXIDATION OF ORGANIC GASES IN ISOTOPIC ANALYSIS

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Continuous Flow Isotope Ratio Mass Spectrometry (CF-IRMS) analysis of gases and compounds is widely used in organic geochemistry. In spite of the fact that the up-to-date level of the CF-IRMS developments is rather high, it needs to be improved and updated.

Accuracy of the resulted isotope analysis data depends essentially on the properties of the being used oxidation reactor capable to oxidize a wide variety of hard oxidizable compounds. Incomplete combustion leads to a considerable distortion of these data.

So, we took an effort to design a simple and reliable oxidation reactor suitable for complete oxidation of organic compounds.

For this particular purpose, we have proposed a novel method of the CF-IRMS analysis based on the oxygen-promoting oxidation of volatile compounds on a surface of platinum electrode of the solid electrolyte reactor (SER). Oxygen penetrates from outside atmosphere throughout the ZrO₂-based oxygen transmitting ceramics at 800-950°C and joins helium flow. The solid electrolyte reactor employed in our device consists of a thin zirconium dioxide ceramic tube and is located right between the standard oxidation and reduction reactors of the CF-IRMS. The design provides a fast change-over from one operating regime to another. Both external and inner surfaces of this tube, coated with porous platinum, serve as electrodes. A source of direct electric current applies the electrical potential to the electrodes. The input of oxygen into the gas-carrier depends on the value of this potential. Since platinum is known to be a perfect catalytic material, the extensive oxidation of compounds being studied takes place on the reactor inner electrode surface at high temperatures.

A mixture of CO₂, CH₄, C₂H₄, C₃H₈, C₄H₁₀ with known carbon isotopes composition estimated by a standard GC-C-IRMS technique has been used as a test sample. The data obtained by our new analytical technique matches nicely the results on carbon isotope ratio measurements carried out by standard oxidation reactor procedure. This confirms the fact of a complete combustion of organic compounds in the new SER we have proposed. Noteworthy, an incomplete combustion leads to an essential error in the isotope ratio measurement.

We have made an actual model of a device designed to develop the optimal conditions of the organic compounds oxidation. The application of our new method involving SER in the CF-IRMS procedure makes it possible to improve the accuracy and precision of isotope analysis. This has been reached due to a relatively small size of the SER as well as to a sufficient amount of oxygen leaked into a helium gas flow and to a well-developed porous catalytic surface of electrode ($<100 \text{ mm}^2$) on which the oxidation takes place. A continuous oxygen flow along with a high temperature regime eliminates a necessity to perform a control over the gas carrier purity and to restore the reactor oxidative capability with no risk of its possible contamination. A long lasting mode of the proposed device work is confirmed by the fact of a numerous practical application cases described for similar solid electrolyte sensors in automobile engines.

Besides, the use of our new SER device allows one to estimate simultaneously concentration and composition of organic gases flowing through the reactor simply from the electric charge transmitted by oxygen ions across the ceramic reactor wall. The sensitivity of our device is very high. At the same time, it is possible to monitor the level of the gas carrier purity.

The data on the organic compounds analysis we obtained are certainly a good reason to consider both method and device as a novel promising approach useful in a wide range of further applications of the CF-IRMS technique.

RECOVERY OF TYRIAN PURPLE FROM ANTHROPOGENIC SEDIMENTS FROM A BRONZE AGE SYRIAN ROYAL TOMB

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The only intact Bronze Age royal tomb to have been discovered in Syria is located at the site of Tell Mishrifeh, formerly the ancient city of Qatna. The tomb is rock cut into a limestone cliff-face and was completely sealed in an undisturbed state following the destruction of the overlying royal palace complex by an invading Hittite army in 1340 BC after *ca* 400 years of continual use (Pfälzner, 2004). The sediments of the tomb floor contained within are purely anthropogenic in nature, having derived from burial activities which took place and the degradation of funerary objects placed within the tomb during its period of use. Our current study is aimed at examining the sediments as a sink of chemical information relating to past ritualistic human activity using a suite of biomolecular and bulk elemental analytical techniques. In several samples, lipid extraction unexpectedly yielded brightly coloured purple extracts, the solubility of which in organic solvents suggested the presence of an organic dye, the most contemporaneous being Tyrian purple.

Negative ion high resolution mass spectrometry of solvent extracts revealed a cluster of ions in the ratio 1:2:1 centred at 418.8853 Da, corresponding to 6,6'-dibromoindigo (**4**), the characteristic coloured component of Tyrian purple (Friedländer, 1909). HPLC-UV/Vis (Figure 1a) revealed a range of brominated and non-brominated indigo and indirubin derivatives (**1-5**) known to be components of Tyrian purple (Karapanagiotis *et al.*, 2006). Preparative HPLC has been used in conjunction with NMR to probe the benzene ring Br substitution patterns of 6-monobromoindigo (**3**) (Figure 1b) and 6,6'-dibromoindirubin (**5**) isolated from the archaeological material.

Tyrian purple was produced on a vast scale from the secretions of the hypobranchial gland of several species of marine mollusc found throughout the Mediterranean. Its association with wealth and royalty throughout its 3000 years of use due to its high intrinsic value is well documented, with at times, the wearing of garments dyed purple being restricted to royalty and priests. The distribution of the dye across the tomb floor is constrained to specific loci and is found in association with the placement of exotic and precious burial

artefacts along with human bones and is likely to have been used to dye textiles which were used in burial or ritualistic ceremonies.

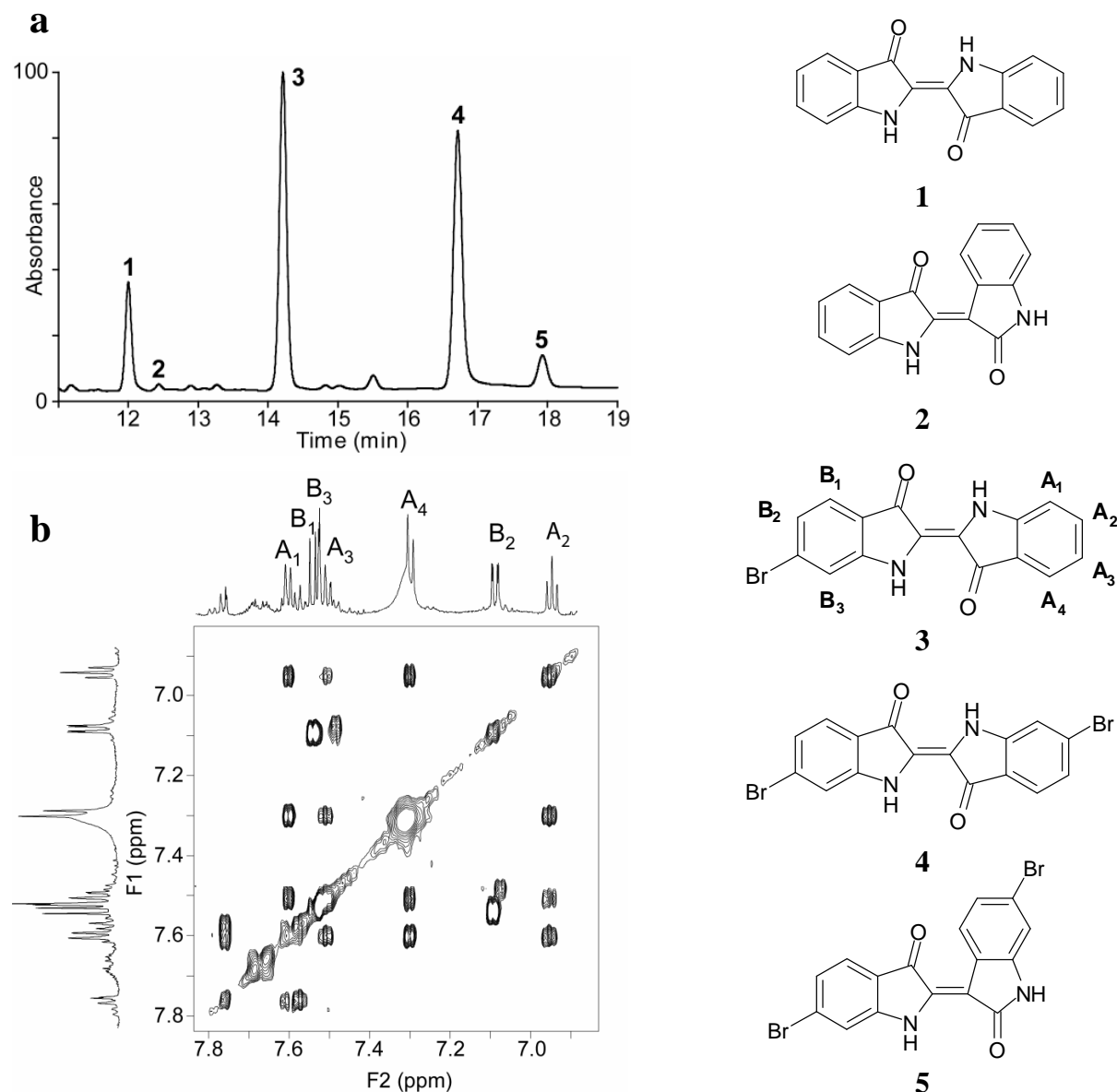


Figure 1. (a) Partial HPLC-UV/Vis chromatogram of DMF extract from sediment sample QS28. (b) 2D ZQ filtered TOCSY NMR spectrum of 6-monobromoindigo (**3**) and assignment of the aromatic substitution pattern. Numbered compounds are as follows; (**1**) indigo, (**2**) indirubin, (**3**) 6-monobromoindigo, (**4**) 6,6'-dibromoindigo, (**5**) 6,6'-dibromoindirubin.

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STABLE NITROGEN ISOTOPE ANALYSIS OF TETRAPYRROLES BY GAS CHROMATOGRAPHY/COMBUSTION/ISOTOPE RATIO MASS SPECTROMETRY

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Stable nitrogen isotopic signature of natural tetrapyrroles such as chloro- and pheo-pigments and alkylporphyrins is a potentially unique proxy for the reconstruction of nitrogen cycle in the aquatic surface environment in the modern to geological past. However, its application has been limited due to the analytical difficulties associated with these molecules. A significant problem is that nitrogen isotope analysis of tetrapyrroles cannot be carried out by gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS) due to low volatility and conjugated cyclic structures of tetrapyrroles, and that alternative isotope analysis by elemental analyzer/isotope ratio mass spectrometry (EA/IRMS) requires a considerable large amount of purified tetrapyrroles (more than micromolar amount of the elements) for the precise determination of isotopic compositions. Therefore, we developed a method to determine nitrogen isotopic composition of tetrapyrroles at nanomolar level, by a combination of chemical degradation treatment of tetrapyrroles into monopyrrole units (*i.e.* maleimides) and isotope analysis of the maleimides by GC/C/IRMS.

Two authentic (pyropheophorbide *a* and mesoporphyrin IX methylester) and four natural (chlorophyll *a*, deoxyphylloerythroetioporphyrin (DPEP) and two 17-nor-DPEP) tetrapyrroles were demonstrated for the isotope measurements of this method. The chlorophyll *a* and DPEPs were isolated and purified from corn leaf and Miocene sedimentary rocks (Onnagawa Formation, Japan) by the improved procedures of Chikaraishi et al. (2005) and Kashiyaama et al. (2007), respectively. These tetrapyrroles were degraded to maleimides by HCl treatment and chromic acid oxidation (*e.g.* Baker et al., 1968; Nomoto et al., 2001) prior to the isotope analysis.

For all authentic and natural tetrapyrroles, nitrogen isotopic composition of the observed maleimides was determined by GC/C/IRMS with a standard deviation (1σ) of better than $\pm 0.5\text{‰}$ at the minimum sample amount of 0.8 nmolN. The isotopic composition determined by GC/C/IRMS is consistent with that of original tetrapyrroles independently determined by EA/IRMS (Fig. 1). These results indicate that no substantial nitrogen isotopic fractionation occurs during the chemical degradation treatment, and that the isotopic

composition of tetrapyrroles can be accurately and precisely determined by the developed method at the sample amount as small as nanomolar level. This method is applicable for nitrogen isotope analysis of natural tetrapyrroles in various biological and geological samples.

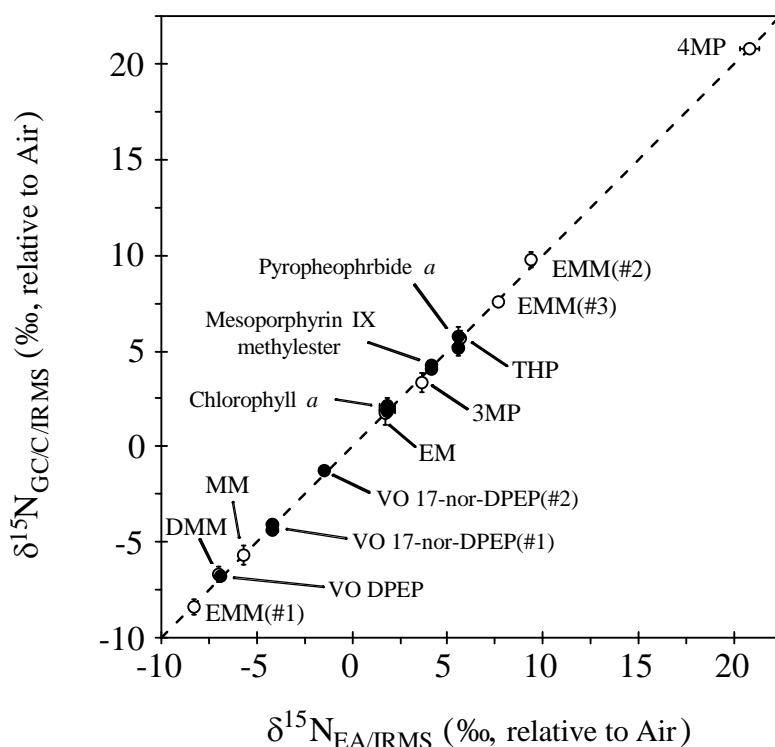


Figure 1. Comparison of nitrogen isotopic composition determined by GC/C/IRMS and EA/IRMS for synthesized maleimide standards (open symbol), and authentic and natural tetrapyrroles (filled symbol). Abbreviation: 2,3-dimethylmaleimide (DMM), 2-ethylmaleimide (EM), 2-ethyl-3-methylmaleimide (EMM), 2-methylmaleimide (MM), 3-Methylphthalimide (3MP), 4-methylphthalimide (4MP), 3,4,5,6-tetrahydrophthalimide (THP), deoxyphyloerythroetioporphyrin complex to vanadium oxide (VO DPEP)

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ANALYTICAL DEVELOPMENTS OF CARBON AND NITROGEN ISOTOPE COMPOSITION IN GULF OF MEXICO SEDIMENTS

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The stable isotope compositions of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) were determined in 37 sediments from the southern Gulf of Mexico by gas chromatograph isotope ratio mass spectrometer (GC-IRMS).

The objective of this work is to discuss briefly the analytical developments of $\delta^{15}\text{N}$ measurements in sediments, the evaluation of the $\delta^{13}\text{C}$ from the saturate fraction, aromatic fractions and bulk of the sediments and compare the organic nitrogen and carbon isotope ratios of organic matter from marine sediments in an attempt to evaluate the technique for its applicability in the geochemistry studies.

$\delta^{15}\text{N}$ provides a powerful tool to trace sources for oceanic and costal production (different nitrate sources), to analyze biogeochemical processes affecting the nitrogen cycle in the sea, and to study trophic chain dynamics (López Veneroni, 1998). Variations in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ ratios have been employed as useful natural tracers to test hypotheses regarding the biogeochemical cycles of nitrogen and carbon in the marine environment (Altabet, 1996).

$\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values varied from -1.0 to 13.0‰ and -27.98 to -20.21‰ respectively. Different distribution patterns of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were observed in the studied sediments. Sediments presenting higher organic carbon values (asphalt-sediments) show $\delta^{13}\text{C}$ values between -28‰ to -27.5‰, which are similar to those observed in some reported Tithonian sourced oils in Mexico (Guzmán Vega and Mello, 1999).

The isotopic composition of the C_{15+} saturate and aromatic fractions is plotted in Fig. 1. The totality of the samples falls below the marine-terrigenous separation line (Sofer, 1984), indicating a marine origin for the samples.

The wide range observed in the $\delta^{15}\text{N}$ values of these sediments could result from the degree of nitrate utilization. The denitrification process in the water column also plays an important role in the nitrogen cycle in sub-oxic conditions. So the enrichment in ^{15}N of some sediments might be caused by water column denitrification.

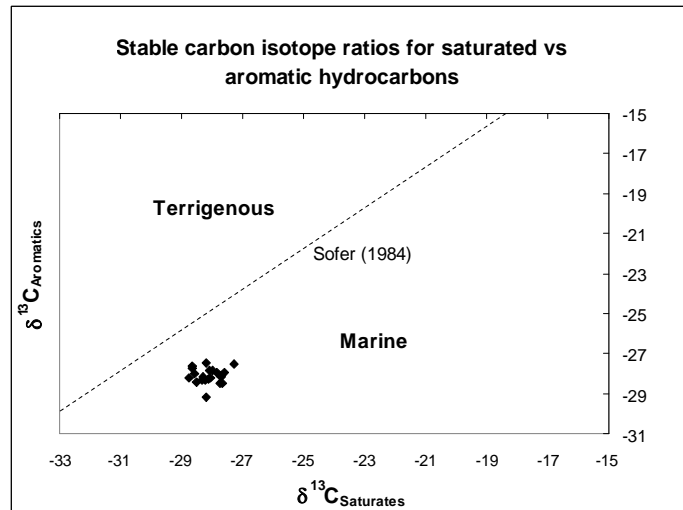


Figure1. Isotopic composition of the C₁₅₊ aliphatic and aromatic fractions. The plot locations suggest that these samples contain mainly marine organic matter.

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APPLICATION OF GC×GC-TOFMS IN THE ANALYSIS OF METEORITIC ORGANIC MACROMOLECULES

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Geological processing has long-since obliterated the Earth-based record of pre-biotic chemical evolution. However, remains of the materials that were involved in the construction of the Earth are preserved in ancient asteroids, fragments of which are naturally-delivered to the Earth as meteorites. Carbonaceous chondrites are a particularly primitive class of meteorite that contain 2 to 5 wt. % carbon, most of which is present as organic matter (Gilmour, 2004).

Off-line pyrolysis and on-line pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) has been used in the analysis of materials such as carbonaceous chondrites (e.g. Watson et al., 2004), however GC-MS systems can be easily overwhelmed by the complexity of the sample and a great deal of information can be lost by the inability to resolve these unresolved complex mixtures (UCM). Recent advances in instrument design have resulted in a major increase in sensitivity and chromatographic resolution.

A Leco Pegasus IV GC×GC-Time of Flight Mass Spectrometry system was used to analyse the organic material in a number of carbonaceous chondrites. The analyses were conducted on an Agilent 6890-5973 GC-MS as well as the GC×GC-TOFMS system which included online pyrolysis, online thermochemolysis and analysis of products released upon off-line hydrolysis.

The main advantages of Py-GC×GC-TOFMS include increased sensitivity of the mass spectrometer over the whole mass range, when compared to the more conventional quadrupole mass spectrometer which is partially due to narrow peaks widths (<0.15 s). Greatly increased signal to noise ratio, due to compounds being separated from the column bleed of the first column on the second GC column. The GC×GC is able to separate compounds that co-elute on a standard gas chromatograph system. Separation of analytes by volatility and polarity enables traditionally unresolved complex mixtures (UCM) to be examined in detail, and vastly increases the number of compounds identified (approximately an order of magnitude increase in resolved peaks). GC×GC is therefore able to afford a greatly enhanced picture of the structural and isomeric diversity of extraterrestrial organic

matter on considerably smaller samples and to provide analyses of samples that were previously unattainable (e.g. rare meteorites, interplanetary dust particles, micrometeorites and returned samples). An example is shown in Figure 1: a contour map of a total ion chromatogram for a pyrolysed sample of Cold Bokkeveld (CM2) macromolecular organic matter. The structural diversity observed in virtually all classes of organic compounds detected, a predominance of branched chain isomers and a decline in abundance with increasing molecular weight are common characteristics that is consistent with the production of meteoritic organic matter through a combination of different processes taking place in a wide range of extraterrestrial environments. (Gilmour, 2004).

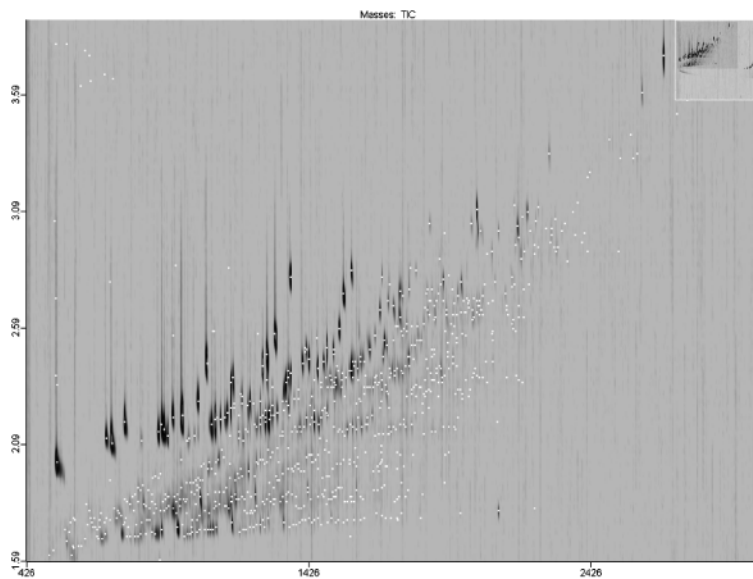


Figure 1. GC×GC total ion chromatogram for hydroxyrolysis products from Cold Bokkeveld.

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MICROFLUIDIC TOOLS FOR ORGANIC GEOCHEMISTRY; RESULTS SO FAR AND FUTURE APPLICATIONS

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A key procedure in organic geochemistry is the separation of complex mixtures of organic compounds into different fractions and this depends on the solubility of different organic compounds within different organic solvents. Take for example the distinctions between kerogen and bitumen or asphaltene and maltene fractions. Many of these fractions are then further separated, until a single type of compound can be resolved, e.g. a maltene fraction is separated into polar and non-polar fractions and these fractions separated further; initially by gas chromatography and then by electron impact ionisation mass spectrometry.

Microfluidic devices comprise small channels, of which one or more has at least one dimension less than 100 μm (figure 1). The use of microfluidic devices as components in lab-on-a-chip and other microengineered systems is now commonplace. Because the channels are small fluid-flow within them is characteristically non-turbulent allowing two fluids to flow adjacent to each other without physical mixing. This would permit the liquid-liquid extraction of dichloromethane by hexane, for example. Furthermore, the same organic solvent may be flown in separate but adjacent streams (using hexane to extract hexane), in this instance compounds will partition between the two fluid phases depending on diffusion rate – which is dependant molecular size (Brody and Yager 1997). Additional advantages of microfluidic devices is that extremely small quantities of sample can be processed and analysed and such processing is potentially very simple to automate.

We have employed microfluidic devices to prepare samples of oil for GC-MS analysis (Bowden et al. 2006), to extract inclusions of organic matter from water soluble minerals and we are developing a lab-on-a-chip procedure to quantify the organic acid content of oils. An important consideration when developing devices is the integration of fractionation (chromatography or a similar separation procedure), derivatisation and analysis stages within a single chip.

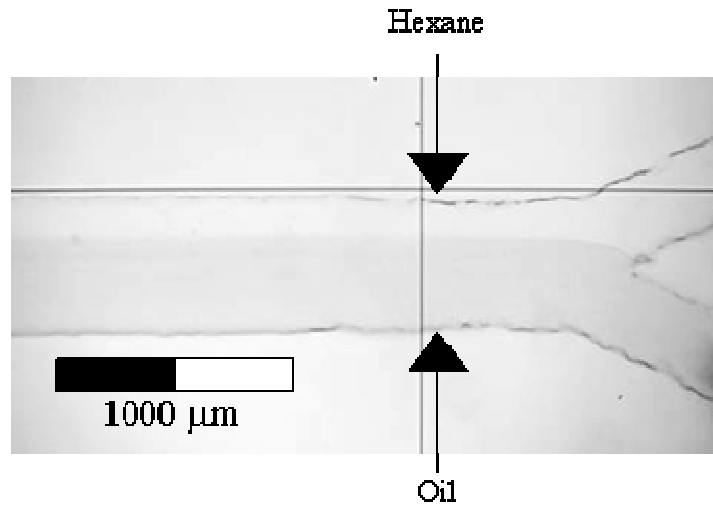


Figure 1. A microfluidic channel etched in glass. Oil and hexane are being flown through a single channel but do not physically mix because the fluid velocity is slow and the dimensions of the channel small. Hexane is being used to extract a hydrocarbon fraction from the oil.

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RAPID NANOMOLAR STABLE ISOTOPE MEASUREMENTS OF NITROGEN AND CARBON FROM ORGANIC SAMPLES

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As part of an ongoing project to measure $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of tetrapyrrole pigments and their derivatives from sediment extracts, we developed a method to decrease sample size requirements for continuous flow elemental analyzer-isotope ratio mass spectrometer (EA-IRMS) systems. Effluent nitrogen and carbon dioxide from the EA are trapped on a silica gel packed column (30cm, 0.75mm ID stainless steel) immersed in liquid nitrogen. The trapped gases are then released in a 1-2 ml min⁻¹ flow of helium by heating to 115°C. Finally nitrogen and carbon dioxide are separated on a carbon PLOT column and introduced to our Finnigan MAT Delta^{plus}XP mass spectrometer through a Conflo III interface. A single sample can be analyzed for both nitrogen and carbon in 12 minutes, making this system preferable to offline trapping methods for small samples.

We minimized the nitrogen background blank from the EA by reducing the inner diameter of the oxidation and reduction furnaces (Carman and Fry, 2002) and the water trap thus decreasing the mixing volume of the system as well as the required trapping time. We also installed a low-flow bleed valve on the EA autosampler to eliminate the effect of leaks associated with the autosampler seals during sample runs. As a result, the nitrogen blank measured on the mass spectrometer has a magnitude of 87±2 nanomoles N₂ and $\delta^{15}\text{N}$ value of -6.13±0.11‰ (vs. N_{air}). Analyses of samples in the 200-400 nanomole N₂ range yielded a precision of 0.2‰ while samples as low as 60 nanomoles N₂ achieved a precision of 0.5‰. The carbon blank is much smaller (6±3 nanomoles, $\delta^{13}\text{C} = -23.95 \pm 1.22\text{‰}$ VPDB) but also less stable in both size and isotopic composition. Most of the organic materials we analyze for carbon and nitrogen have high C/N ratios so the carbon blank is at least 2 orders of magnitude smaller than the sample and thus does not significantly affect the $\delta^{13}\text{C}$ measurements. With improved carbon blank, we anticipate sample sizes for carbon that can be run in the absence of the helium dilution from the Conflo III interface.

These new modifications reduced the minimum sample size requirement from ~1000 nanomoles N₂ on the conventional EA system to 60 nanomoles N₂ on our “nanoEA” system. This ~15-fold decrease in sample size enables compound-specific coupled $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements on large molecules such as chlorophyll *a* that are incompatible with GC-IRMS.

The presentation will focus on isotopic measurements of whole extracts and pigment fractions collected after separation using a high performance liquid chromatography quaternary solvent gradient system (Airs et al., 2001).

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INVESTIGATION OF PHOTOSYNTHETIC BACTERIA AND ALGAE BY RESONANCE RAMAN SPECTROSCOPY

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Cyanobacteria and algae comprise a large and diverse group of organisms that are widely distributed in freshwater, marine, terrestrial, and extreme environments. Cyanobacterial and algal identification and classification rely on morphological characterization and 16S rRNA gene sequence analysis. Morphology however, can be misleading in certain circumstances, while 16S rRNA gene sequencing can be time consuming. Analysis of pigments that have taxonomic value may be an alternative approach to the identification, classification, and discrimination of cyanobacteria and algae in environmental samples.

It is well-known that the chloroplasts of photosynthetic organisms contain a wide variety of pigments which in turn are known to be taxonomic markers (Fawley, 1989; Andersen et al., 1996). The primary biological function of these pigments is the absorption of light. The light energy is then either transferred to photosynthetic reaction centres to drive photosynthesis or re-emitted at longer wavelengths, to avoid photodamage to cells. These pigments fall into three main categories: chlorophylls and carotenoids, both soluble in organic solvents; and phycobiliprotein which is soluble in water. There are several types of chlorophyll, including chlorophyll *a*, *b*, *c1*, *c2*, *c3*, and *d*. All appear green. In contrast there are more than 60 carotenoid pigments found in cyanobacteria and algae which range in colour from orange to red. Some carotenoids, such as β -carotene and zeaxanthin, are widely distributed throughout algal groups, whereas others are highly specific to individual groups. Phycobiliproteins are unusually found in cyanobacteria, red algae, and a small group of flagellates (Trainor, 1978).

If a biological molecule absorbs light in the visible portion of the electromagnetic spectrum, then new possibilities are opened up for Raman spectroscopy of these compounds of interest, via the resonance Raman (RR) effect. Judicious selection of tuning the excitation wavelength to the electronic absorption spectrum can produce selective enhancement of certain Raman bands. These Raman bands correspond to vibrational modes which involve motions of the atoms in the chromophore, being that portion of the molecule where the electronic transition is localised. Therefore, RR spectroscopy provides a means whereby

vibrations of biological chromophores can be distinguished from many of the vibrational modes associated with the complex biological matrix. Significantly, the resonance enhancement factor can be quite large, in the order of 10^3 to 10^6 orders of magnitude, thereby allowing the analysis of chromophore concentrations as low as 10^{-4} to 10^{-6} M.

A total of nine photosynthetic bacteria and micro-algae were studied by RR spectroscopy ($\lambda_{\text{excitation}}$ 488 nm). The cyanobacteria investigated include *Nostoc sp*, *Anabaena sp*, *Microcystis sp*, Antarctic isolated cyanobacteria, and Yellowstone National Park isolated cyanobacteria. The micro-algae investigated include two Chlorophyta green micro-alga, namely *Botryococcus braunii* Race A and B and two Dinophyta, namely *Lingulodinium machaerophorum* and *Tuberculodinium vancampoae*. All spectra contain major features at approximately 1005, 1155, and 1525 cm^{-1} , which are strongly resonance enhanced due to carotenoids. These bands at 1500-1500 cm^{-1} and 1150-1170 cm^{-1} are due to in-phase C=C (ν_1) and C-C stretching (ν_2) vibrations of the polyene chain. Additionally, in-plane rocking modes of CH_3 groups attached to the polyene chain coupled with C-C bonds occur in the 1000-1020 cm^{-1} region. The most significant spectral change observed was the position of the C=C (ν_1) band. The position of the ν_1 band is strongly dependent on the length of the carotenoid chain (Withnall et al., 2003; Veronelli et al., 1995).

The position of the ν_1 band acquired from the RR spectra of the cyanobacteria and algae follow the trend: Peridinin carotenoid (*Lingulodinium machaerophorum* and *Tuberculodinium vancampoae*) > Botryoxanthin carotenoid (*Botryococcus braunii* Race A and B) > β -Carotene (*Nostoc sp*, *Anabaena sp*, *Microcystis sp*, Antarctic isolated cyanobacteria, and Yellowstone National Park isolated cyanobacteria). RR spectroscopy has shown to be a useful rapid and non-destructive tool in the discrimination of photosynthetic bacteria and eukaryote micro-algae based on carotenoid analysis.

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SURFACE ENHANCED RAMAN SPECTROSCOPY: A TOOL FOR THE SEARCH FOR LIFE ON MARS

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A relatively inexpensive optical fibre based raman system was assembled with of the shelf components, to study the benefit of surface enhanced raman spectroscopy (SERS) as a possible tool for the detection of biologically significant UV screening pigments such as scytonemin found in cyanobacteria. Cyanobacteria living in gypsum are considered to be a good terrestrial analogue for possible extant (or extinct life) on Mars (Edwards, Villar et al. 2005) where its surface is exposed to significant amounts of UV-A, B and C solar radiation. Scytonemin in particular is interesting as it can act as a UV-C filter, which is thought to be a remnant from an environmental adaptation for early life on Earth. Samples of colonies from microbial infestations of gypsum were taken from the Haughton impact crater on Devon Island. Micro litre extracts were obtained from milligram samples of the bacteria inhabited gypsum and were then analysed using surface enhanced raman spectroscopy (SERS), employing an Ocean Optics QE65000 spectrometer with a spectral resolution of 8cm^{-1} . The SERS signal provided by the system was found to be linear down to 10nM concentrations scytonemin. The enhancement obtained by SERS was of the order 10^6 for scytonemin (a major pigment found in cyanobacteria colonies recovered from the Haughton impact crater). Although, the resolution of the spectrometer could not resolve all the peaks reported (Edwards, Moody et al. 2005) for scytonemin, this pigment was easily identified by the system. This study has shown the effectiveness of SERS to produce an inexpensive, readily assembled and yet extremely sensitive sensor for possible extraterrestrial biomarker for life detection.

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USING LASER MICROPYROLYSIS TO DECIPHER THE CHEMICAL RECORD OF METAZOANS DURING THE “CAMBRIAN EXPLOSION”

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Representatives of nearly all the animal phyla living on Earth today made their first appearance in the fossil record near the base of the Cambrian (ca. 542 Ma). Considerable effort has been directed in recent years to elucidation of the apparently rapid radiation of metazoans during the “Cambrian Explosion” bioevent (e.g. Marshall, 2006). However, this research is challenged by the fact that conventional palaeontological studies are mostly limited to preserved hard parts of organisms and to trace fossils. Thus, relatively little information is available on the evolution of many soft-bodied organisms (~70% of typical metazoan biota), or the soft-parts of fossilised biota, due to the rapid decay of such tissues during the process of fossilisation. In contrast, some molecular residues of organisms survive the normal taphonomic processes of decay (e.g. Skinner, 2005) and can accumulate in host sediments. For example, it has been suggested that many of the soft bodied fossils from Early to Middle Cambrian Konservat-Lagerstätten deposits are preserved as organic (kerogenised) films resulting from interaction between clay minerals and the original lipids derived from the organisms (Butterfield, 1995).

As part of a wider palaeontological and organic geochemical study of the cryptic pre-history of major metazoan groups, laser micropyrolysis gas chromatography–mass spectrometry has been applied to an Early Cambrian shelly fossil. This technique allows 10–100 µm spot size analysis (Greenwood *et al.*, 1998), so spatially-selective chemical data can be acquired from different regions in a single fossil and the surrounding taphonomic residues. Initial experiments were carried out on chemically-isolated and hand-picked shells of the stem group brachiopod taxon *Askepasma* (Holmer *et al.*, 2006), from the Early Cambrian Wilkawillina Limestone, Flinders Ranges, South Australia. The calcium phosphate shells were ultrasonicated in dichloromethane prior to analysis. Organic-rich rocks such as the Sydney Basin torbanite laboratory standard pyrolyse at relative low powers (e.g. 10 x objective, 10A for 1 sec: 0.5 W). For *Askepasma*, laser power had to be increased (12.5A for 1 sec: 7.2 W), and multiple shots (5 to 10) were aggregated on the cold-trap. Under these

conditions, laser micropyrolysis results were obtained that were significantly above the system blank for the instrument. Pyrolysis products include *n*-alkanes (Fig. 1), *n*-alkenes, alkylnaphthalenes, alkylphenanthrenes and other polycyclic aromatic hydrocarbons. Laser micropyrolysis on separate *Askepasma* shells gives reproducible data, with some variations, for example in the *n*-alkane molecular weight distribution. These initial data suggest that intracrystalline kerogenous material is preserved within the calcium phosphate matrix of the Cambrian shells, as has been documented for modern brachiopods (Curry *et al.*, 1991). Further experiments will aim to elucidate the kerogen composition and will investigate its origin, which could be from the original brachiopod, and/or from fungal or bacterial degradation products.

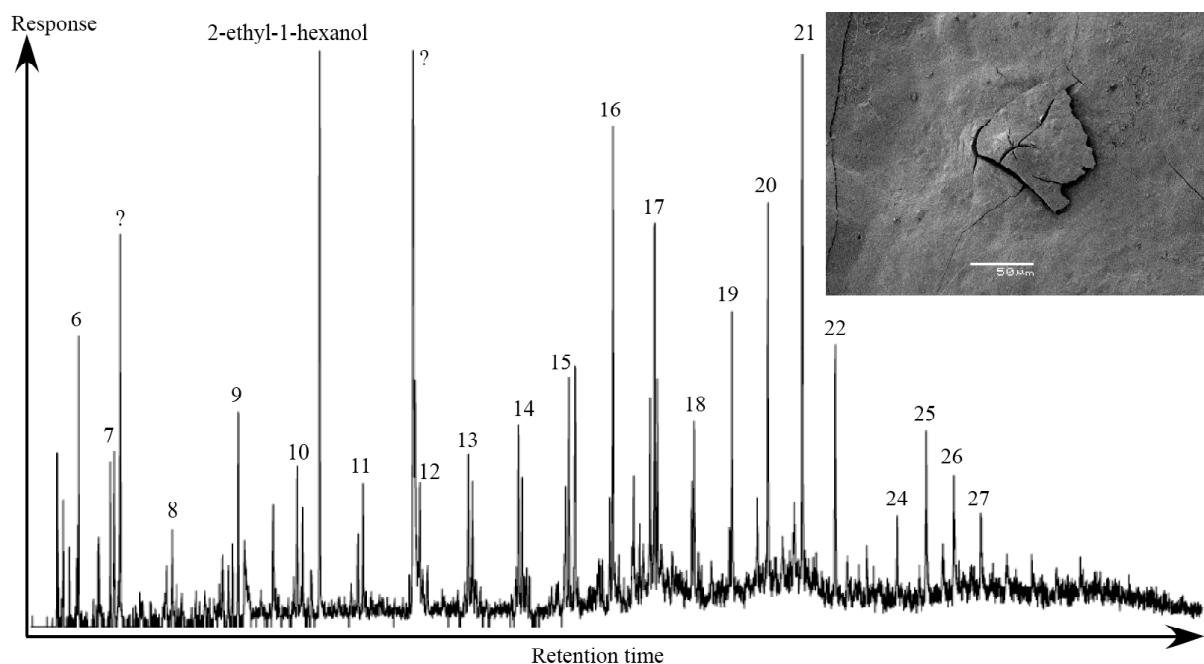


Figure 1. Mass chromatogram (m/z 57) of the laser micropyrolysis of an *Askepasma* shell (five laser shots aggregated), and SEM micrograph of one laser crater. Numbered peaks are *n*-alkanes.

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INSIGHTS FROM PROTEIN-SPIKED ARTIFICIAL SOILS INTO THE VALUE OF MATRIX-ASSISTED LASER DESORPTION/IONIZATION MASS SPECTROMETRY BASED SOIL PROTEOMICS AS TO INTERPRET ARCHAEOLOGICAL SITES

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Anthropogenic activities are known to leave chemical traces in the soil, primarily in the form of organic molecules released from occupation waste. Recent studies indicate that lipids, fatty acids and waxes can survive in soils for long periods of time and show the potential of these residues as archaeological biomarkers. Although some of these biomarkers can be traced back to their origin, the information yield is often limited, because many organic molecules in the soil have multiple sources, become mineralized or are heavily degraded. Proteinaceous biomarkers on the other hand hold specific information about anthropogenic action on the soil, but are generally believed to be very susceptible to degradation especially on archaeological time scales.

Although soils are one of the most abundant archaeological artefacts, investigation of soil proteins is quite limited. Studies on recent soils nevertheless suggest that proteins can be strongly retained by means of adsorption onto clay minerals, encapsulation by refractory organic matter, protein complexation or various combinations of these processes. Soil composition and resulting characteristics (e.g. pH, cat/anion-exchange capacity) are thus of great importance for the long-term preservation of proteins and choice of method to extract and identify proteins. Previous soil proteomic studies have focused mainly on protein extraction using strong acids or bases, chaotropic agents and phosphate buffers, whereas quantification and identification were assessed using protein assays (i.e. Bradford assay) and immunological methods respectively. Although these approaches give an indication of protein concentration and type, they are often biased by the presence of co-extracted non-Proteinaceous substances.

In this study, retention experiments with bovine serum albumin (BSA) and heat treated BSA were carried out with four protein extractants (hydrofluoric acid, guanidine-HCl, citrate and phosphoric acid/bicarbonate) and eight artificial soils containing different amounts of quartz sand, clay minerals (bentonite and kaolinite) and organic matter (humic acid and glucose). Also, matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS)

based soil proteomics were applied to both artificial and archaeological soils in order to i) study how co-extracted soil constituents affect protein detection and identification and ii) assess the value of such an approach for the interpretation of archaeological sites.

Results from this study indicate that levels of extracted proteins from soils are a function of both extractant and soil type and that the protein domains that are involved in absorption processes are highly dependent on soil type. In turn, this affects the efficiency of the applied proteomic approach, because of the limited amounts and different kinds of protein fragments that are available for SDS-PAGE separation, proteolysis, MALDI-MS analysis and protein identification. However, from most artificial soil extracts abundant proteinaceous signals could be obtained as to successfully identify BSA and this shows the potential of soil proteomics as an advanced method for archaeological prospection and site interpretation. In contrast to these findings, extraction experiments with archaeological soils implied that proteinaceous signals in these soils are often quantitatively and qualitatively insufficient for protein identification. This is probably due to the low concentration and degradative state of proteins in archaeological soils.

A UNIVERSAL PASSIVE SAMPLER FOR DISSOLVED ORGANIC MATTER IN FRESHWATER AND MARINE ENVIRONMENTS

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Dissolved organic matter (DOM) represents the largest pool of mobile carbon on Earth, and is a fundamental link between the terrestrial and aquatic environments. DOM is a significant contributor to the global carbon cycle, an important mediator in the physical and chemical interactions that govern the fate and transport of many contaminants, and an integral player in climate change. However, despite its importance, the structural components in DOM have yet to be described in detail, due in part to the difficulty in its isolation. In this study, we first describe the use of a novel passive sampler for the isolation of DOM from freshwater, show the limitations of this passive sampler in saline waters, and present an improved design for the universal application in both freshwater and marine environments.

The freshwater passive sampler consists of a molecular weight selective membrane (1,000 kDa) and an anion exchange resin (diethylaminoethyl-cellulose (DEAE-cellulose)) (Fig.1). Nuclear Magnetic Resonance (NMR) spectra indicate the samplers isolate DOM that is near indistinguishable from that isolated using the batch DEAE-cellulose procedure. However, the material isolated on the passive samplers cost ~\$0.15 per mg to isolate while DOM isolated using the traditional batch procedure costs \$8-10 per mg. Samplers are easy to construct, negate the need for pressure filtering and also permit a range of temporal and spatial experiments that would otherwise be difficult or impossible to perform using conventional approaches. For example, DOM can be monitored on a regular basis at numerous locations, or samplers could be set at different depths in large lakes. They can also be deployed into hard to reach environments such as wells, ground water aquifers, etc, and as they are easy to use, they can be mailed to colleagues or included with expeditions to isolated places such as the Arctic and Antarctic.

The samplers have been shown to be effective in a range of freshwater environments including a large inland lake (Lake Ontario), a fast flowing tributary, and a wetland. However, due to the high affinity of Cl⁻ ions for the DEAE-cellulose, samplers that employ this resin are not effective in saline environments. To overcome this limitation, new resins have been examined as a substitute for a universal passive sampler that can be used in both fresh and saline waters. Here we discuss preliminary results of a passive sampler employing

activated charcoal as a selective resin for DOM sorption and show its effectiveness as a universal passive sampler (see Figure 1).

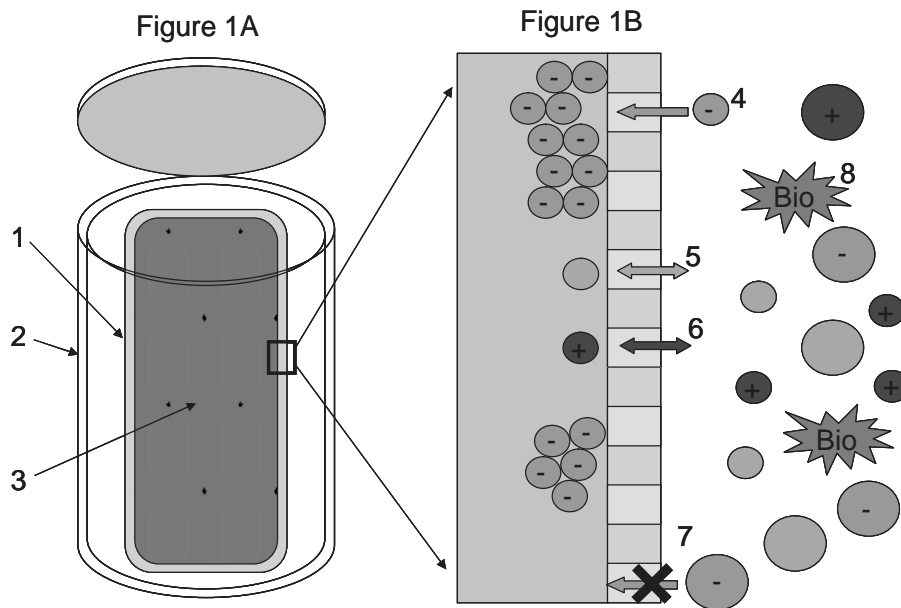


Figure 1A. Diagrammed schematic showing the components of the passive sampler. 1) 1,000 kDa Molecular Weight Cut Off (MWCO) Poly(vinylidene fluoride) (PVDF) membrane. 2) porous high density polyethylene (HDPE) casing to house sampler unit (designed in house) to prevent large organisms (fish etc.) and debris from compromising the membrane. 3) resin for DOM sorption. **Figure 1B.** Expanded region showing the resin/membrane/water interface. 4) dissolved negatively charged DOM enters the membrane and are sorbed onto the resin and concentrated many fold, 5+6) dissolved neutral or positively charged species (for example, metals in the case of positively charged) can enter the membrane but are not retained (*note* the vast majority of DOM is negatively charged), 7+8) large species including particulate organic matter and biological species cannot enter the membrane. The use of the membrane removes the need for filtering.

MEMBRANE PROCESSES APPLIED TO THE CHARACTERIZATION OF MARINE ORGANIC MATTER

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Natural organic matter (NOM) is a complex and heterogeneous mixture of macromolecules, which are the degradation products of plants, animals and microorganisms. The characterization of NOM is difficult, since its composition depends on climatic, geographical and biological conditions. The study of marine organic matter is even more challenging, due to the low concentration of organic substances in seawater (less than 1 mg/L) and the presence of salts in significant amounts. The objective of this work was to combine several membrane processes (ultrafiltration, nanofiltration, reverse osmosis and electrodialysis) to better characterize marine organic matter from the Gironde estuary (South Western France) and from the Mediterranean Sea (Toulon, South Eastern France).

Dissolved organic matter (DOM) was first fractionated by ultrafiltration (UF) in order to separate DOM components in function of their molecular size. The fractionation was achieved by using sequentially three membranes of decreasing molecular weight (MW) cut-offs: 3000, 1000 and 500 Da. Each fraction was then analyzed by spectrofluorometry (more precisely 3D excitation-emission matrix spectroscopy), which presents the advantage to be a technique sensitive to very low concentrations of fluorescent molecules. By combining UF and fluorescence spectroscopy, we were able to determine more precisely the size distribution of DOM fluorophores and thus the type of compounds present in marine organic matter. As for the dissolved organic carbon (DOC) analyses of the different fractions, they showed that our marine samples were mainly constituted of small molecules (MW lower than 1000Da).

At the same time, we developed a process which allows a fast and efficient concentration of marine organic matter while minimizing the salt content of seawater samples. This concentration step is necessary in order to obtain sufficient amounts of DOM and then to carry out quantitative analyses such as NMR, IR or GC/MS. Reverse osmosis (RO) is now largely used to isolate DOM from large volume of freshwaters (Serkiz *et al.*, 1990; Sun *et al.*, 1995), because DOM is not modified during RO and the yields in DOC are high (generally more than 80%). But to date, a paper dealing with the concentration of marine organic matter

by RO has not yet been published. The reason is that during RO only water is removed by the membrane and therefore salts are concentrated exactly like the organic matter. Therefore, seawater must be desalted prior the concentration of DOM by reverse osmosis.

In a first time, a nanofiltration (NF) membrane was used for the desalting step. Thus, 940L of Mediterranean seawater were desalted and reduced to 32L by NF and then concentrated to 10.5L by RO. According to fluorescence analyses, DOM was not significantly modified during both NF and RO steps, since the 3D spectra of the NF and RO retentates (concentrated samples) were roughly similar to the one of the initial seawater sample. Losses of DOM during the concentration by RO were negligible. Even though a large amount of organic matter was lost during the NF step, we finally obtained a significant concentrated DOM sample representative of the initial seawater organic matter. In order to improve concentration yields, another method was considered to desalt seawater before (or after) the concentration by RO.

Electrodialysis (ED) appeared as an interesting alternative in order to remove salts and not organic matter from seawater. ED is a gentle electrochemical technique, allowing the separation of ionic species from aqueous solutions by using an applied direct current potential across ion-exchange membranes. 20L of Mediterranean seawater were desalted from 37g/L to 3 g/L in less than 7 hours by using an ED stack with a total surface membrane area of 0.2 m² and by applying a constant voltage of 14V. The DOC content of the diluate (desalted seawater) stayed approximately constant throughout the experiment (about 1 mg/L). Therefore, the combination of ED and RO could be a method of choice to isolate and concentrate marine organic matter without fractionating it, as it is the case when XAD resins or UF membranes are used.

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A FAST METHODOLOGY FOR THE PREPARATION OF THE SEAWATER SOLUBLE FRACTION (SWSF) OF THE FUEL OIL

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Many operational problems have been described in the literature related to the preparation of the sea-water soluble fraction (SWSF) from oil mixtures in the laboratory. There is not a standard procedure which gives comparable and reproducible results (e.g. Ziolli and Jardim, 2002). Until now, practically all the SWSF preparation methods have been carried out in low energy-mixing systems, generally using magnetically stirring. This approach is time consuming, and the dissolution experiment can last several weeks. Here we describe a new method for the preparation of SWSF. This is based on a high energy system using ultrasonication which shortens the dissolution study to just a few days until equilibration is reached.

The experimental set up requires stirring oil in an ultrasonic bath for 30 minutes and a settling period of 48h. The system was held in darkness to avoid photodegradation. Water samples were recovered through a PTFE tub to monitor the progress of the dissolution. This was quantified using ultraviolet fluorescence spectroscopy (Ali et al., 1995). A diesel oil solution was used for the calibration of the fluorescence measurement. Deuterated standards were used to control recoveries at the end of the experiment and for quantification. The identification and quantification of the main individual hydrocarbons of the SWSF was carried out by GC-MS (gas chromatography-mass spectrometry).

Appraisal of the method has been done using a fuel oil sample (bunker C) mixed with water at different temperatures and salinities. The method has proven to generate reproducible SWSF. As expected, light polyaromatic hydrocarbons (PAHs) such as naphthalene and its alkyl derivatives, C1, C2 and C3-naphthalenes, were found to be the most abundant compounds in the SWSF (Figure 1). Their concentration in SWSF rises as a function of increasing water temperature and decreasing salinity.

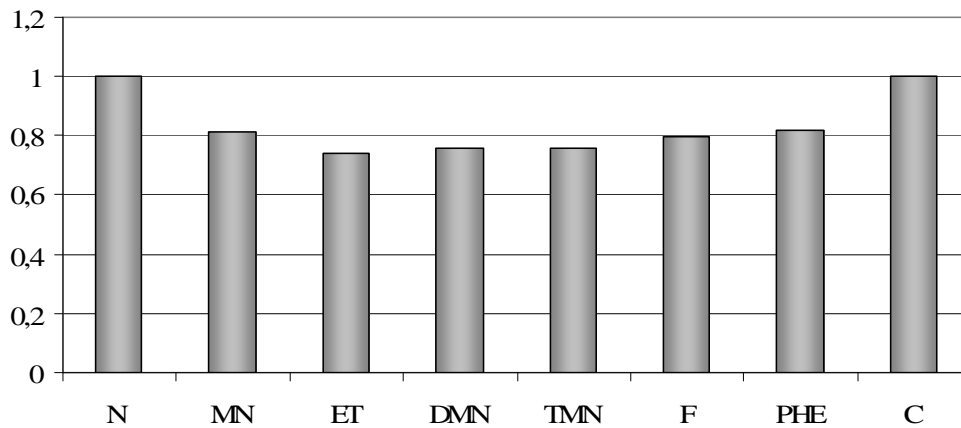


Figure 1. Relative abundance of the major light PAHs founded in the SWSF. N: Naphthalene; MN: Methylnaphthalenes; ET: Ethylnaphthalene; DMN: Dimethylnaphthalenes; TMN: Trimethylnaphthalenes; F: Fluorene; PHE: Phenanthrene; C: Carbazole.

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HYDROPYROLYSIS AS A PREPARATIVE METHOD FOR THE COMPOUND SPECIFIC CARBON ISOTOPE ANALYSIS OF STEROIDS

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The ability to accurately determine the carbon isotopic composition of steroids by standard gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) techniques would be of great benefit for a variety of environmental and biological science applications. However, steroids in their natural form exhibit poor chromatographic resolution, while derivatisation adds extra carbon atoms thereby corrupting the starting stable isotopic composition of the target molecules (Wolthers and Kraan, 1999).

This study describes the application of hydroxylation to the defunctionalisation of individual steroids to yield their corresponding hydrocarbons, thus retaining the carbon skeleton intact while improving chromatographic resolution, allowing for the faithful measurement of carbon isotope ratios. Hydroxylation, which involves the catalytic addition of hydrogen to the carbon skeleton under a high hydrogen gas pressure (15 MPa), was originally developed as a method for the analysis of covalently bound biomarkers in crude oils and source rocks (Love et al., 1995), and has been successfully used to defunctionalise fatty acids prior to GC-C-IRMS analysis (Sephton et al., 2005a).

The product recovered after the hydroxylation of 5α -cholestanol (Fig. 1a) is composed largely (>95%) of 5α -cholestane. Very small amounts of 5β -cholestane and two isomers of unsaturated cholestene were also detected. The results illustrate that, in addition to defunctionalising aliphatic chains as demonstrated for carboxylic acids (Sephton et al., 2005a), hydroxylation also efficiently eliminates exocyclic oxygen-containing functional groups. For cholesterol, effective hydrogenation would be expected to produce two cholestane isomers (5α and 5β) owing to the non-selective nature of the hydrogenation reaction for alkene moieties adjacent to positions where rings join. However, GC/MS analysis of the products (Fig. 1b) shows that extensive rearrangement occurs with the hydroxylation procedure giving, in addition to the two expected cholestane isomers, four diasteranes and an unresolved complex mixture, comprising other diasteranes, cholestenes and diasterenes.

Comparison of the carbon isotopic determinations for untreated 5α -cholestanol and cholesterol (as determined by combustion-IRMS), and the products of hydroxylation by GC-

C-IRMS (Fig. 1), indicate that the isotopic composition of the processed sample is consistent with that of the starting material. The difference between the two measurements is within the error of the instruments and it appears that no isotopic effects are associated with conversion from the functional steroid to their hydrocarbon counterpart.

Poor selectivity was observed for other multifunctional or unsaturated steroids such as testosterone and androstanediol. Therefore, developments to the catalyst system and temperature regime are ongoing in order to promote low temperature hydrogenation, and so eliminate rearrangements caused by the migration of the carbon double bonds, thereby achieving the highly selective defunctionalisation and accurate isotopic characterisation of these analytically challenging molecules.

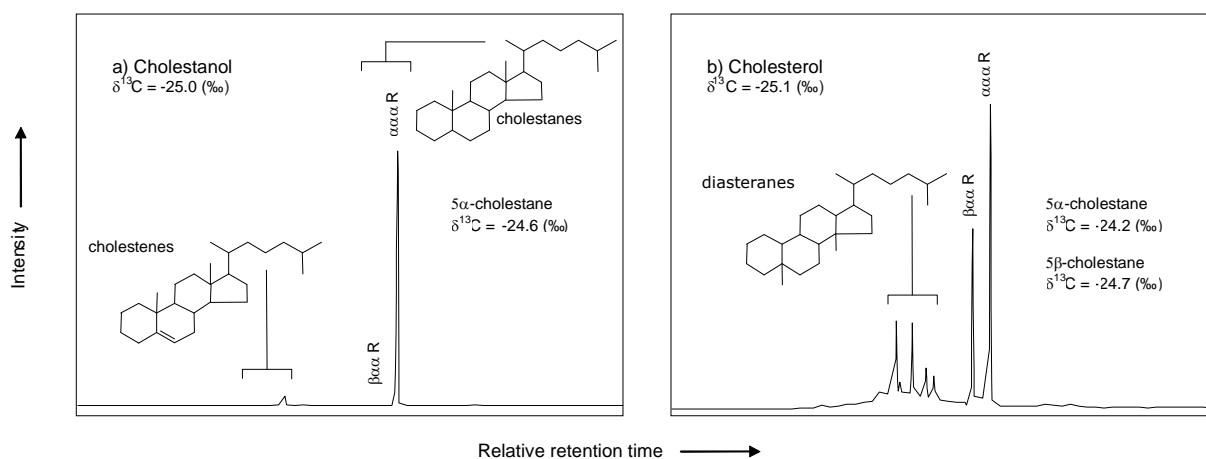


Figure 1. GC-MS traces of the hydroxyprolysis products of (a) cholestanol and (b) cholesterol (modified from Sephton et al., 2005b).

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ENRICHMENT OF STERANES AND HOPANES BY MOLECULAR SIEVE NaX AND CaX FOR COMPOUND SPECIFIC ISOTOPE ANALYSIS

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Compound Specific Isotope Analysis (CSIA) is a useful method in geochemistry, it can be used for reconstruction of paleoenvironment (Summons and Powell, 1987) and oil-oil and oil-source rock correlation (Guthrie et al., 1996). To carry out CSIA accurately, analysis of the entire peak without co-elution of other compounds is required, and during preparative separation of organic compounds without isotopic fractionation of individual compounds is also necessary (Bidigare et al., 1991). Molecular sieves are shape-selective separation materials which have no fractionation effect during separation of organic compounds (M.Schoell et al., 1992, Kening et al., 2000, Moldowan and Dahl, 2004). In this study, we establish a method using column chromatography packed with molecular sieve NaX and CaX to separate hopanes and steranes, and biomarkers CSIA can be realized.

The procedure of the method includes the following main steps:

1) Silica gel is used for separation of saturated hydrocarbon from crude oil or source rock extract.

2) Molecular sieve ZSM-5 is used for remove of n-alkanes from saturated hydrocarbon, and the branched and cyclic fractions are obtained.

3) Place the branched and cyclic fractions on the top of glass column filled with molecular sieve NaX, use pentane to rinse it till it wets half of the column.

4) Rinse the column with pentane after 30 minutes. Collect the pentane elution and concentrate it in a rotate evaporator.

5) Dry the column with N₂. Pour out the molecular sieve NaX and extract it with isooctane in a small type of Soxhlet extractor for more than 30 hours. The hopanes are obtained, as shown in Figure 1 A0.

6) Place the concentrated elution got from step 4) on the top of glass column filled with molecular sieve CaX, use pentane to rinse it till it wets half of the column.

7) Rinse the column with pentane after 30 minutes. Collect the pentane elution 5 cuts. Steranes of different configurations and gammacerane, β -carotane are separated or enriched, as shown in Figure 1 A1 to A5.

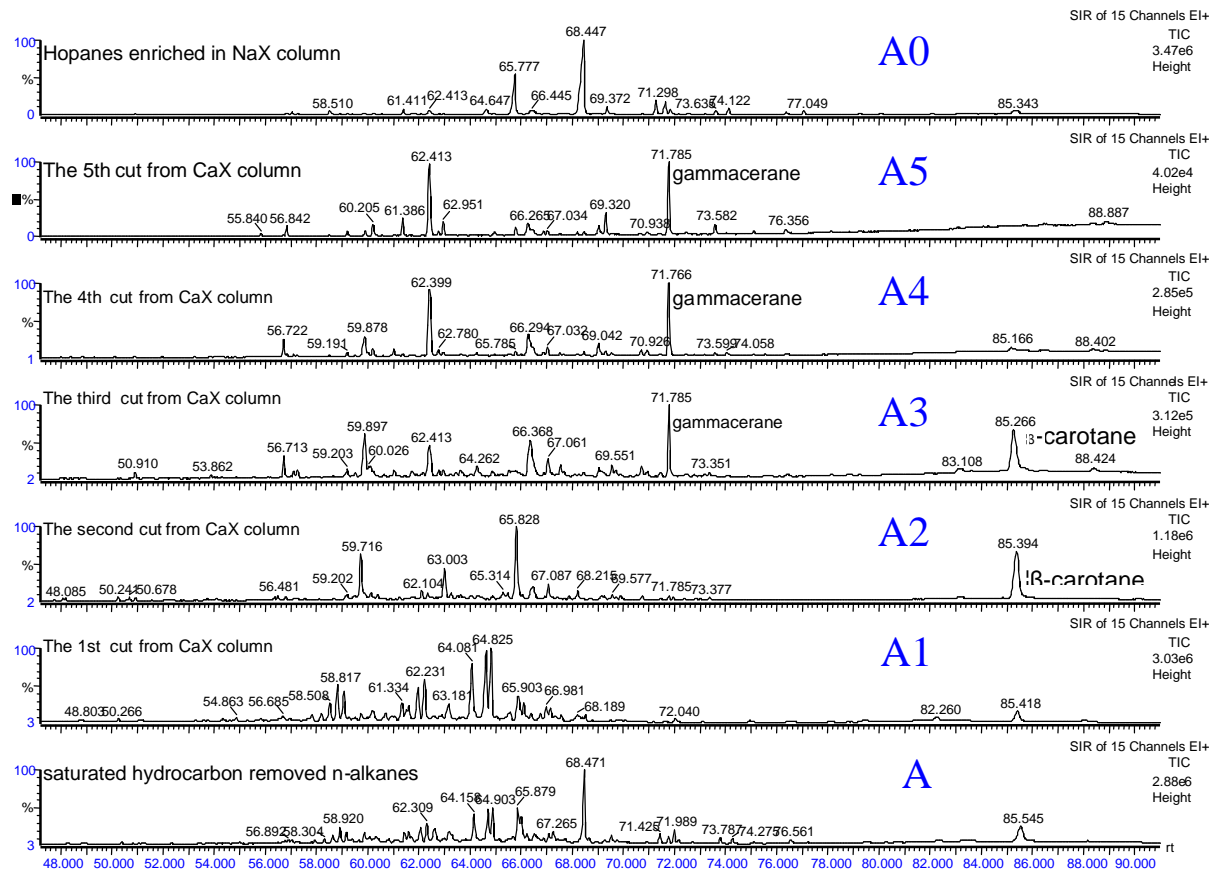


Figure 1. Total ion chromatogram for each fraction by GC/MS. A-saturated hydrocarbon removed n-alkanes, A1-A5, each 3ml elution from molecular sieve CaX, A0-hopanes enriched in molecular sieve NaX.

Compounds are identified by interpretation of mass spectral data and comparison with available standards and data in the literature. Compound Specific Isotope Analysis in fraction A0-A5 can be carried out by GC-C-MS.

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ANALYSIS OF PHYTOL, ALCOHOLS, CUTIN ACIDS AND STEROLS IN MARINE AND LAKE SEDIMENTS BY ON-LINE TMAH/THERMOCHEMOLYSIS GC-MS

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On-line TMAH/thermochemolysis GC-MS analysis (TMAH method) consists of thermally-assisted hydrolysis and *in situ* methylation with tetramethyl ammonium hydroxide (TMAH) reagent. We have previously reported a rapid analytical method of lignin phenols and alkanolic acids for small amount of sediment sample (Yamamoto and Ishiwatari, 2005). We report here the result of examining an optimum condition for analyzing compounds with hydroxyl group (e.g. alcohols, sterols etc.) in a sediment sample using TMAH reagent.

A Curie point pyrolyzer (used as a TMAH reaction chamber) directly connected to GC-MS instrument was used in this study. We examined an influence of TMAH reaction temperatures on the yield of derivatization of compounds with hydroxyl group. We used marine (offshore California) and lake (Lake Baikal) sediments for this study. Typically, 5-7mg of dry fine powdered sediment sample is placed in a pyrofoil (ferromagnetic plate) and 20 μ l of a 25% TMAH methanol solution and internal standard (e.g. *n*-C₁₉ fatty acid) was added. After methanol was evaporated to dryness, the pyrofoil was set into the pyrolyzer and heated at various temperatures (315, 358, 445, 500, 590, 670°C) for 20 seconds. The TMAH products were analyzed by GC-MS.

Major TMAH reaction products from the sediment are phytol methyl ether (plus its underivative), cutin acid derivatives, *n*-alcohol methyl ethers (plus their underderivatives), sterol methyl ethers and *n*-C_{22:1} amide (plus *N*-methyl and *N,N*-dimethyl derivatives), other than *n*-C₁₈-C₃₀ fatty acids methyl esters (Fig. 1). A possible source of 11,18-diOH C₁₈ acid is marine organisms (Yamamoto et al., 1992). The other compounds are typical biomarkers, e.g., phytol from chlorophyll from phytoplankton, *n*-C_{22:1} and *n*-C_{24:1} alcohols and *n*-C_{22:1} fatty acid from zooplankton or shrimp (e.g., Yunker, et al. 1995). 9, 16- or 10, 16-diOH C₁₆ acid from cuticle of leaves (e.g., Gôni and Hedges 1990), cholesterol from zooplankton or algae, brassicasterol from diatoms, and dinosterol from dinoflagellate (e.g., Volkman 1986).

The yields of all compounds are apparently high at TMAH reaction temperatures of 315-445 °C as compared with higher temperatures (>500 °C) (Fig. 2). At higher reaction temperatures, the amounts of underivatized compounds for phytol and *n*-C_{22:1} alcohol are high. Therefore, the optimum TMAH reaction temperature is concluded to be 315-445 °C for phytol, fatty alcohols, sterols and cutin acids, in so far as the present reaction system is concerned. The maximum amounts of sterols obtained by this TMAH method at 358 °C are considerably higher than those obtained by a solvent extraction method (2.0-7.3 μ g/g-ds vs. 1.7 -3.8 μ g/g-ds for offshore California sediments).

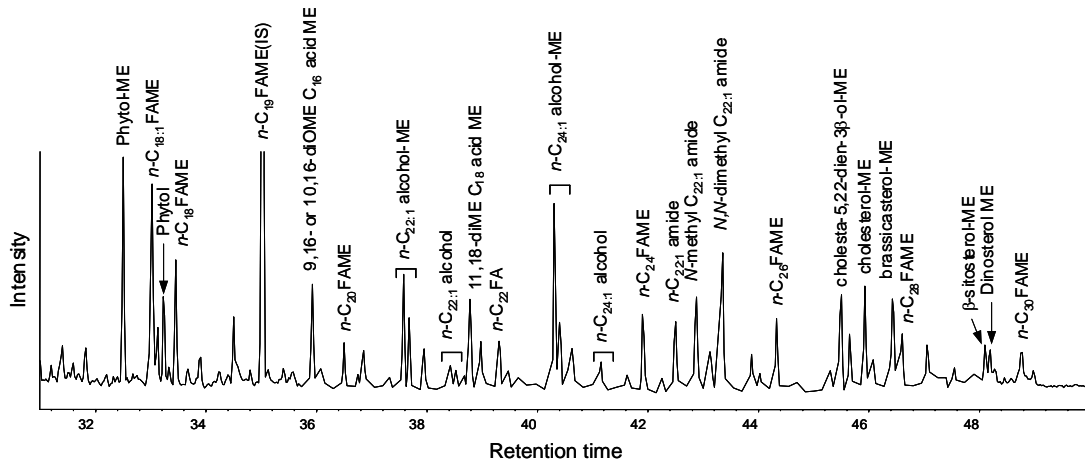


Figure 1. Total ion chromatograms (TIC) and the major organic compounds produced by the on-line TMAH/thermochemolysis from the California offshore sediment. Abbreviation: FAME: fatty acid methyl ester, n:carbon number, IS:internal standard, ME: methyl ether and methyl ester derivatives.

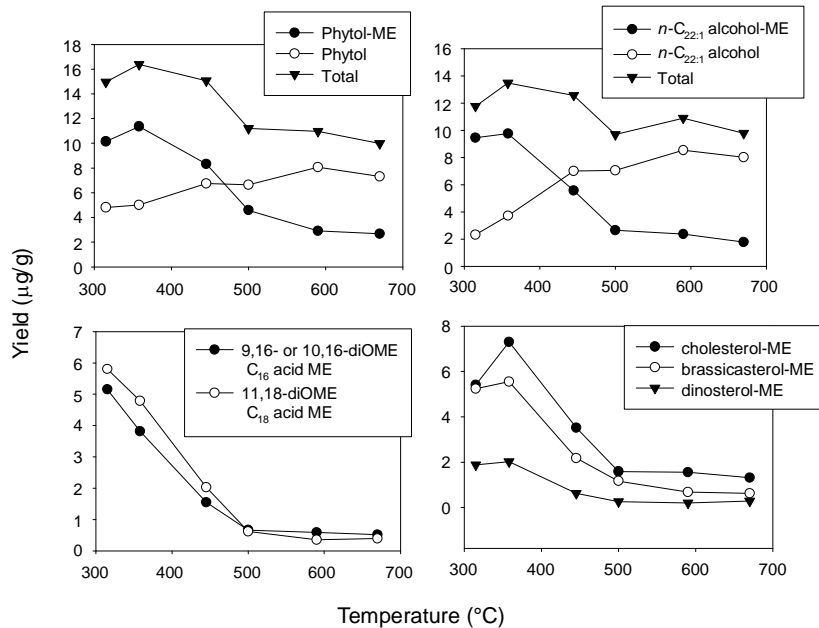


Figure 2. Influence of temperature for the on-line TMAH/thermochemolysis on the yields of phytol, n -C_{22:1} alcohol, cutin acids and sterols from the marine sediment. ME indicates methyl ether and methyl ester derivatives.

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A RAPID AND NON-DESTRUCTIVE FOURIER TRANSFORM NEAR-INFRARED SPECTROSCOPY (FT-NIRS) METHOD TO RECONSTRUCT ORGANIC CARBON, CARBONATE AND OPAL RECORDS FROM MARINE AND LACUSTRINE SEDIMENTS

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Total organic carbon (TOC), CaCO₃ and Opal are bulk geochemical parameters representing a major share of most aquatic sediments and also coal beds. Analysing all three components traditionally requires separate analytical methods and intense sample preparation which are time consuming and expensive if performed at high depth resolution and over long study sections, typically required for high-quality climate records. Near Infrared Spectroscopy (NIRS) has been known for over 100 years, although recent enhancements in technology has meant that this method now has routine applications in environmental science, medicine, agriculture and pharmaceuticals amongst others. Fourier transform near-infrared spectroscopy (FT-NIRS) is a fast, non-destructive and inexpensive technique with the potential to provide accurate key elemental data on natural samples and thus partly replace wet chemical analyses in marine and climate research (e.g. Chang et al., 2005). Here we expand on this approach and report the application of FT-NIRS to a wide range of natural sediments including modern and Quaternary marine sediments, Eocene lacustrine deposits, and Cretaceous black shale.

The marine sediments used in this study were taken from the Equatorial Atlantic and covered deep sea, continental margin, river fan, upwelling and Cretaceous black shale environments. Samples from the Equatorial Atlantic were chosen not only for their wide diversity but also for their importance in understanding tectonics, global carbon budgets as well as past and present climate systems. Lake Messel was chosen as a paleolacustrine setting since it has over one million years of consistent sedimentation and also the presence of a kerogen Type I oil shale with exceptionally high TOC concentrations.

Analyses were performed on a Nexus 870 FT-IR/FT-NIR spectrometer, fitted with a Smart UpDrift accessory (ThermoNicolet Corp.) which allows measurements on powdered samples in diffuse reflection mode, with a quartz sample window to reduce scatter effects. Analyses were carried out over a wavelength range of 875-2500nm at 1nm resolution, yielding 1625 raw data points per sample. Although near infrared spectra display characteristic absorption peaks and troughs relating to specific vibrations from molecular

bonds, their complex, broad overlapping nature relating to overtone and combination bands means that multivariate statistics are needed to relate absorptions at specific wavelengths with chemical data of interest. In this study we used Partial Least Squares Regression (PLSR) as a numerical technique to calibrate and, importantly, predict element concentrations. All data were subject to various spectral pre-treatments to determine the best calibration equations, defined by the lowest RMSEP and the highest R^2 value.

The calibrations developed show strong predictive ability for all three constituents ($R^2 > 0.9$) in all of the different settings, providing evidence that FT-NIRS has wide potential for the rapid analysis and prediction of element data of a very wide range of sediments. As one example we show the results for TOC measurement in Figure 1. The plot shows a fully internally cross validated calibration set of conventionally measured (using a Leco CS244 instrument) versus FT-NIRS predicted TOC data using 405 samples from all study sites covering a range of TOC concentrations from 0.04 % to 41 % wt.

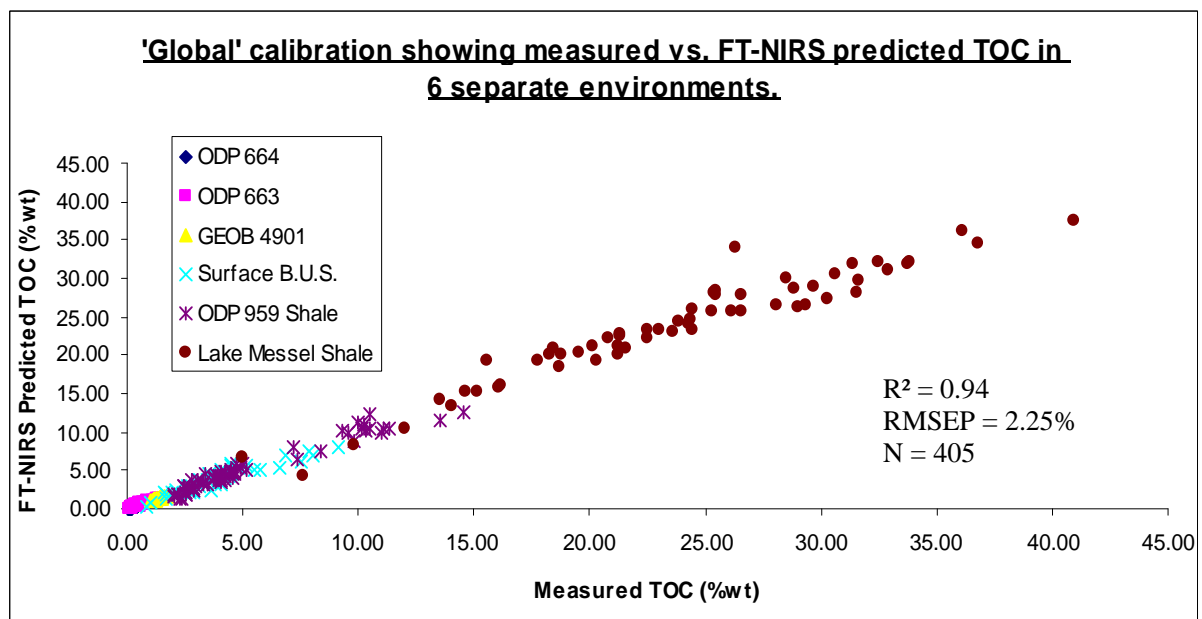


Figure 1. Comparison of measured (Leco) and predicted (FT-NIRS) TOC data for a range of marine and lacustrine sediments. Modern: Benguela Upwelling System; Quaternary: ODP 663/664 and GeoB 4901; Eocene: Lacustrine Messel oil shale (courtesy P. Hofmann, Cologne); Cretaceous black shale: ODP 959

We conclude that this technique has the potential to be developed into a fully automated non-destructive scanning technique providing a novel tool for a broad range of environmental and marine applications, in particular for high-resolution climate research and petroleum geology.

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**CURIE-POINT PYROLYSIS / GAS CHROMATOGRAPHY / MASS
SPECTROMETRY OF STANDARD DIPEPTIDES IN THE PRESENCE OF
TETRAMETHYLAMMONIUM HYDROXIDE**

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Nitrogen-containing organic compounds represent the second most abundant reservoir of nitrogen on earth. Despite the important implication of nitrogen in environmental processes, its chemical structure and origin remain widely unknown likely due to the refractory nature of its source macromolecules (30 to 50% of total nitrogen in humic substances) (Schulten & Schnitzer, 1998). Recent studies (del Rio *et al.*, 2004; Knicker & Hatcher, 2001), using ^{15}N NMR spectroscopy, pointed to the dominance of amide functions in natural environments, thus suggesting that N is engaged in polar building blocks. Pyrolysis in the presence of tetramethyl ammonium hydroxide (TMAH) has appeared as a powerful tool for the study of nitrogen-containing moieties.

For a few years, TMAH-Py/GC/MS has been applied to the study of refractory OM from different sources, and focused on nitrogen-containing macromolecules. For a better understanding of the pyrolysis results of these materials, the pyrolytic behaviour of model compounds has been investigated (Hendricker & Voorhees, 1998; Zang *et al.*, 2001). Thus the twenty protein amino acids have been pyrolysed in the presence of TMAH and a data base has been constituted (Gallois *et al.*, submitted).

As nitrogen mainly occurs in higher molecular weight structures and not as free amino acids, the influence of the peptidic linkage on the pyrolytic behaviour of amino acids has been investigated and eighteen dipeptides have been studied. TMAH-pyrolysis of dipeptides revealed that they do not behave as the mixture of the corresponding amino acids. In the pyrochromatogram of dipeptides the major pyrolysis products of free amino acids are generally only detected at low levels, except for the aromatic derivatives of the aromatic amino acids which remain very important. The most abundant compounds correspond to higher molecular weight derivatives, thus, methylated diketopiperazines as well as methylated derivatives of the dipeptides have been regularly identified at the end of the chromatograms. A good example of this is given by the pyrolysis of Ala-Tyr, presented in fig. 1.

Moreover, to precise the role of the peptidic bond on the pyrolytic behaviour of the amino acids, pyrolyses of symmetrical pairs of dipeptides were performed. They demonstrated the importance of the functional group involved in the peptidic bond. Compared

to high molecular weight derivatives the amount of pyrolysis products from one amino acid is enhanced when the amino acid is C-terminal.

So, as far as we can conclude, the pyrolysis of dipeptides in the presence of TMAH yields mainly stable cyclic structures (DKP and aromatics for corresponding amino acids) and also releases methylated derivatives of dipeptides in relatively important amounts.

Further work on higher molecular weight peptides and proteinaceous material will now take advantage of the amino acids data base and these considerations on dipeptides.

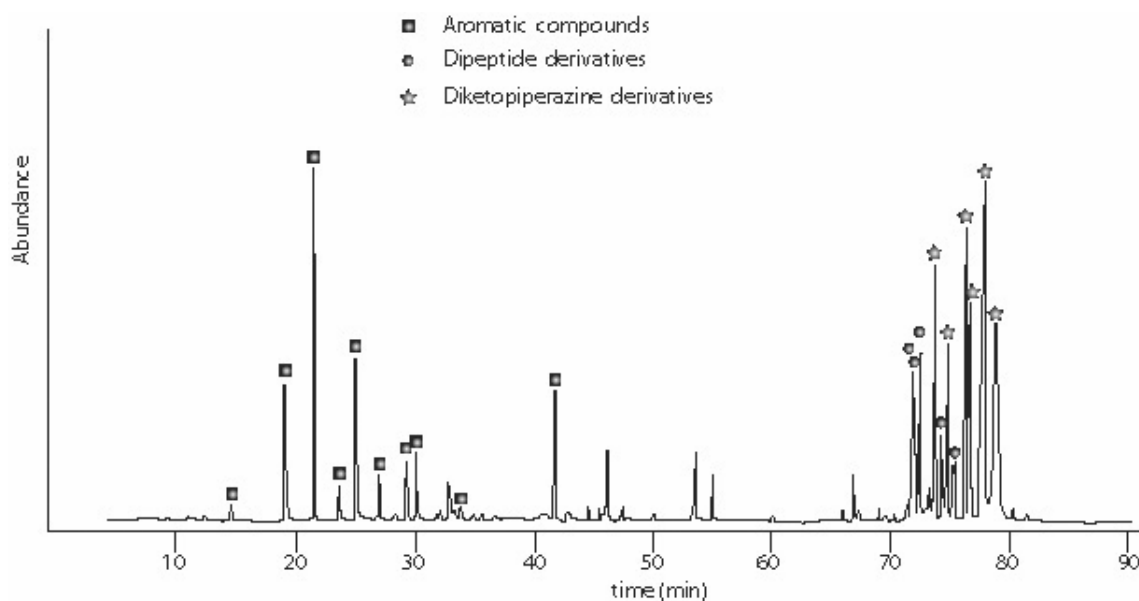


Figure 1. TIC chromatogram of the TMAH pyrochemolysis products released from Ala-Tyr.

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EXPLORING GEOCHEMICAL DATA USING NON-LINEAR PROJECTION METHODS

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Exploration, often using visualisation methods is a well established practice in statistics and geochemistry. However, most methods that have been applied to date are based on the concepts of 'linked plots, brushing and sample sets' or linear projection methods, typically principal components analysis or some form of multi-dimensional scaling.

In this work we show how non-linear projection methods, that preserve either the data topology ('Generative Topographic Mappings') or data geometry ('Neuroscale'), can be applied to geochemical data sets. For example we show how such 2 dimensional non-linear projections can be used to visualise, interpret and classify the similarities between oils from different wells using a range of geochemical properties. We contrast the non-linear methods to more traditional linear methods, discussing the strengths and weaknesses of each when applied to geochemical data, and in particular oil-oil and oil-source rock correlations in petroleum exploration. We show how to tackle the common problem of missing data in an optimal Bayesian framework and show the effect of this on visualisation. Finally we discuss how such a non-linear visualisation tool could be deployed in a commercial setting.

TOTAL SULFUR IN ROCKS AND KEROGENS AS A PART OF COMPLEX GEOCHEMICAL EXAMINATIONS - PRECISION AND VALIDATION STUDY

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Total sulfur in rocks, kerogens, oils and bitumens is a small but significant part of complex geochemical examinations. Determination of the total sulfur is, by definition, part of the ultimate analyses of rocks (cores and cuttings) serving a number of interests: combined with a major element analysis it amends the chemical composition useful in the process of petrologic determination of the rock mineral matrix. Total sulfur in oil is significant for physical and chemical characterization. Sulfur content classifies oils as high and low sulfur types.

Organic geochemical investigations include determination of the total sulfur content in a soluble organic matter (EOM) and in kerogen as well. Optical analyses of the organic matter determine primarily the type of kerogen and its maceral composition, but also a stage of thermal maturity. Organic petrologic examinations estimate the presence of pyrite in the organic matter but quantitative determination of sulfur confirms the results of optical analyses. Total sulfur content points to specific a kerogen structure. High percentage of sulfur in kerogen enables generation of hydrocarbons even in a diagenetic stage of thermal evolution as a result of the preferential cleavage at the weak sulfur linkages. Presence of pyrite suggests anoxic deposition environment conditions as well as presence of heavy metals which bond with sulfur in sulfides.

This work presents a test report, analytical precision and validation study of the sulfur determination on the coal and rock reference materials as well as the sulfur determination on the core, cutting and kerogen samples from Palmyra, Jihar, Al Bahra, Al Mahr and Mudawara exploration wells in Syria, referring the requirements of the standard test method ASTM D 4239-05 for conformity evaluation of the sulfur determination in Geochemical Laboratory:

ASTM D 4239-05 is the test method for sulfur in the analysis of coal and coke samples using high-temperature tube furnace combustion method, while our samples are mostly cores, cuttings and kerogens. Silicate rocks are inherently heterogeneous materials, since they are composed of discrete minerals of diverse chemical composition and physical character. The ultimate limitation of analytical precision is therefore the homogeneity of the

rock, as well as the complex nature of kerogen. That makes the sulfur analysis in rocks and kerogen a demanding analysis, according to the ASTM D 4239-05 standard test method requirements. Some of the uncertainties regarding the sample homogeneity effects are overcome by the rock crushing specified by ASTM D 4239-05. Those rock samples should be milled to pass completely a No 60 (250 μ m) mesh sieve or even a No100 (150 μ m). Good repeatability values are sometimes hard to obtain on the kerogens and rocks with a high content of sulfur. It seems that enlarged amount of vanadium pentoxide (0.8 to 1.0 g for approx. 0.50 g kerogen or rock sample), as combustion aid substance during analysis, improves results and repeatability values of the analysis.

Good accuracy and precision of the sulfur analysis in rocks and kerogens, using high-temperature tube furnace combustion method, are strongly based upon the analysis of the reliable, specific reference materials related to the nature and to the range of sulfur values of the materials to be tested. The accuracy requirement according to ASTM D 4239-05 test method for coal and coke can be achieved, under defined conditions, in the analysis sample of rocks and kerogen.

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