Journal of Chromatography, 356 (1986) 147–162
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 365

SOME ANALYSES OF LIPID CLASSES IN MARINE ORGANISMS, SEDIMENTS AND SEAWATER USING THIN-LAYER CHROMATOGRAPHY–FLAME IONISATION DETECTION

J. K. VOLKMAN* and D. A. EVERITT
CSIRO Division of Oceanography, GPO Box 1538, Hobart, Tasmania 7001 (Australia)
and
D. I. ALLEN
Department of Chemistry, University of Tasmania, GPO Box 252C, Hobart, Tasmania 7001 (Australia)
(First received July 8th, 1985; revised manuscript received November 27th, 1985)

SUMMARY

Applications of the silica gel Chromarod-Iatroscan TH-10 thin-layer chromatography–flame ionisation detection (FID) system for identifying and quantifying the non-polar lipids in a variety of marine samples are presented. Excellent results can be obtained with a speed and sensitivity which is lacking in other analytical procedures. However, the non-linearity of the FID response at the low loadings (<2 μg) required for most samples of interest makes the use of FID calibration curves mandatory. Seawater and sediment samples contain large amounts of polar lipids, degraded organic matter and pigments which can also interfere with the lipid analyses. Lipid compositional data are presented for the unicellular algae *Thalassiosira eccentrica* and *Emiliania huxleyi*, the marine copepods *Paralabidocera antarctica* and *Calanus finmarchicus*, seawater particulate matter from northern Tasman Sea waters off the East Australian coast and sediments from Ace Lake, Antarctica and the Great Barrier Reef of Australia.

INTRODUCTION

Thin-layer chromatography (TLC) combined with flame ionisation detection (FID) is now widely recognized as a useful technique for identifying and quantifying lipid classes in organic materials[1–5]. Its use as a quantitative method has been questioned[6], but acceptable results can be obtained provided that features inherent in the technique are understood. These include different response factors for various classes[3,4,7–9], variations caused by Chromarod-scanning parameters[12], humidity effects on R_F behaviour[3], Chromarod variability and changes with extended use[3,13,14], and the choice of method used to apply the sample to the Chromarod[15].

The flame ionisation detector is approximately linear at loadings up to 100 μg[14,16,17], but at low loadings (<1–5 μg), a curved response is observed for some compounds[13]. To date, most applications of TLC–FID have been in the fields of

0021-9673/86/$03.50 © 1986 Elsevier Science Publishers B.V.

medical research and comparative biochemistry where information on the amounts of specific lipids in extracts of tissues or organs is required[1,4,8,9,16,18,19]. There have been few attempts to apply the technique to the analysis of complex lipid mixtures which occur in the natural environment, such as in seawater[3,13], marine organisms[19,20] and sediments[22–24], despite the obvious need for such a method. For these, strategies based on multiple developments of the Chromarods in different solvent systems are often necessary[2–4]. In marine samples, the lipid mixtures can consist of hundreds of compounds and usually one has to detect and accurately quantify only a few micrograms or less of any particular lipid class.

In this paper we present examples which highlight some of the strengths and weaknesses of the TLC–FID method for studying the lipid compositions of marine organisms, sediments and seawater.

EXPERIMENTAL

Samples

Results are presented for lipid analyses of phytoplankton, seawater particulate matter, zooplankton and sediments which are representative of the major types of samples likely to be of interest to those studying marine lipids.

The diatom *Thalassiosira eccentrica* (isolate CS-17) was obtained from the culture collection of Dr. S. W. Jeffrey (CSIRO Marine Laboratories, Cronulla, Australia) and cultured in f/2 medium at 17.5°C for 21 days under a 12 h light–12 h dark cycle. The coccolithophorid *Emiliania Huxleyi* was cultured at the laboratory of the Marine Biological Association (U.K.) in f/2 medium as previously described[25]. The algae were filtered through glass fibre filters and washed briefly with saline solution before extraction. The copepod *Paralabidocera antarctica* was obtained from net hauls in Ace Lake, Vestfold Hills, Antarctica in February 1984[23]; approximately 20 animals were analysed. Total lipids from the copepod *Calanus finmarchicus* from the North Sea were donated by Dr. J. R. Sargent, NERC Institute of Marine Biochemistry, Aberdeen, U.K.

Surface sediment from Ace Lake, Vestfold Hills was obtained from a core collected in June, 1978 from the centre of the lake[23]. This sediment is organic rich, anoxic and contains abundant remains of the alga *Pyramimonas gelidicola*. The coral reef sediment was collected in July, 1984 from Davies Reef (18°48′S, 147°39′E) in the central section of the Australian Great Barrier Reef, about 70 km ENE from Townsville, Queensland. The water depth at the time of collection was 4 m. This sediment is oxic and contains low concentrations of organic matter derived from a diversity of coral reef animals, plankton and bacteria. Both sediments were kept frozen at −20°C until analyzed.

Seawater particulate matter was collected during cruise SP11/83 of R/V Sprightly in July, 1983 from a station in the Tasman Sea (32°S, 155°E), 220 km north-east of Sydney. Water samples were collected at 19.00 h from 1 m, 30 m, 60 m, 90 m and 120 m using 8-l Niskin bottles, filtered immediately on-board ship and then stored in liquid nitrogen until analysis. Estuarine samples were collected at Battery Point, Hobart, Tasmania in February, 1985.

A similar extraction scheme was followed for each sample with modifications as required. Samples of biota and seawater particulate matter were extracted with

propan-2-ol (7 ml, 10 min) using ultrasonication, and then chloroform–methanol (2:1, 3 × 10 ml). The propan-2-ol extraction was included to minimize transesterification which can occur in the presence of chloroform:methanol. To remove salts and water soluble material, the combined extracts were partitioned with reagent-grade water from a Milli-Q water system which contained an Organex-Q cartridge designed to remove organic material (Millipore). They were then filtered through a glass fibre filter (GF/C, Whatmans), evaporated to dryness using a rotary evaporator, and dissolved in chloroform–methanol (2:1, 1 ml) for TLC–FID analysis. Wet sediment ($\approx$10 g) was extracted with propan-2-ol (20 ml, 10 min) and then with chloroform–methanol (2:1, minimum of 4 × 20 ml) until the extracts were pale yellow in colour. Each extract was centrifuged (3–5 min, 350 *g*), combined and then worked up as above.

Apparatus and operating conditions

The equipment consisted of an Iatroscan Mk III TH-10 TLC–FID analyzer (Iatron Laboratories, Japan), Shimadzu Chromatopac CR2A-X integrator/plotter and YEW 3056 2-pen recorder. The flame ionisation detector was operated with a hydrogen flow-rate of 160 ml/min and an air flow-rate of 2000 ml/min. Scan setting 4 was used corresponding to a speed of 0.42 cm/s. The chart recorder was operated at a speed of 20 cm/min and a sensitivity of either 0.5 or 1.0 m*V*/cm.

Silica gel SII Chromarods (5 μm particle size) were kept overnight in a humidity tank and passed through the detector twice before use. Samples were applied using a 5-μl syringe (S.G.E., Australia) or 0.5- and 1-μl disposable micropipettes (Drummond). The syringe was found to be superior for most applications due to its larger volume capacity and ease of operation. The concentrations of lipids in many samples was so low that a single application of the sample using a micropipette did not transfer sufficient material to the rod. Multiple applications using a micropipette were tedious and could produce broad starting bands. Focussing techniques can be used[26], but, in practice it was easier to apply the required volume of sample (up to 10 μl) in a single slow application using a syringe.

Chromarods were developed in glass tanks lined with pre-extracted filter paper. A number of solvent systems were investigated and the most useful for the analysis of non-polar lipids was hexane–diethyl ether–acetic acid (60:17:0.15). For polar lipids, chloroform–methanol–water (80:15:2) was used. Hexane–diethyl ether (96:4) was used to separate wax and steryl esters from hydrocarbons when required. After the Chromarods were developed they were oven dried for 5 min at 100°C and analysed immediately, to minimize adsorption of atmospheric contaminants. The Chromarods were regularly cleaned by immersion in chromic acid overnight (typically after 10 analyses) followed by a 5-min wash in running water and a final rinse in redistilled water.

Precautions against contamination during sample collection

During the collection of marine samples there is a high potential for contamination especially from plastic materials (silicones and phthalate esters) and from the lubricants and fuels used on ships (hydrocarbons). Further contamination can be introduced during laboratory work-up of the samples from solvents and chemicals. The formation of degradation products from pigments and labile lipids during sample

manipulations must also be avoided since these degradation products are also formed in the natural environment. These difficulties are exacerbated by the low concentrations of lipids which usually occur in marine sediments and seawater.

To minimize these problems, sample manipulations were kept to a minimum. Seawater was filtered through two previously extracted glass fibre fine filters (GF/F) but only the top filter which did not contact the filtering manifold was analyzed for particulate matter. Samples were immediately stored in liquid nitrogen to minimize degradation of lipids and pigments. Procedural blanks were performed regularly; contamination with hydrocarbons and phthalate esters during sampling and filtering can be a major problem when working on ships and stringent precautions need to be taken to minimize contamination. Two common phthalate esters have similar mobilities on silica rods to triacylglycerols and wax esters respectively depending on the choice of solvent system; these were resolved from triacylglycerols using the solvent system hexane–diethyl ether–acetone (94:4:4)[3], and from wax esters using hexane–diethyl ether–acetic acid (94:6:0.2).

RESULTS AND DISCUSSION

In most studies of marine lipids, concentration data are presented for only a few lipid classes. Capillary gas chromatography combined with mass spectrometry is presently the most useful method for identifying constituents within a lipid class, but the technique is very time consuming if more than one or two lipid classes are studied. High-performance liquid chromatography provides an alternative technique for separating lipid classes in a single analysis but the lack of a suitable universal detector for lipids is still a major limitation preventing its more widespread use in lipid analysis. The ability of the Iatroscan TLC–FID technique to both separate and detect complex mixtures of lipids at the low concentrations found in environmental samples makes it potentially of great value for studies of marine lipids. However, peculiarities and limitations inherent in the technique need to be considered before reliable data can be obtained.

Quantitative aspects

Quantitative accuracy and reproducibility is still a major weakness in the TLC–FID system. The FID response of most lipids is significantly different from that which would be calculated from their ionizable carbon content, partly due to losses during volatilisation before ionization and other factors[6,10,12]. One consequence is that the FID response of each compound class must be determined experimentally. Also, some data[6] indicate that compounds within a lipid class may give different FID responses depending on their chain-length. Other structural features such as degree of unsaturation may also have an effect but as yet this has not been tested.

A common approach is the use of "correction factors" calculated by comparison of the FID response with that of a lipid standard such as triacyl glycerol or ketone. However, inconsistencies between literature values are common. Delmas *et al.*[13] recently showed that the FID responses of several compounds were non-linear at low loadings. The cross-over point between 2 linear fits to the data for high and low loadings were between 1 and 5 μg depending on compound class. We have studied

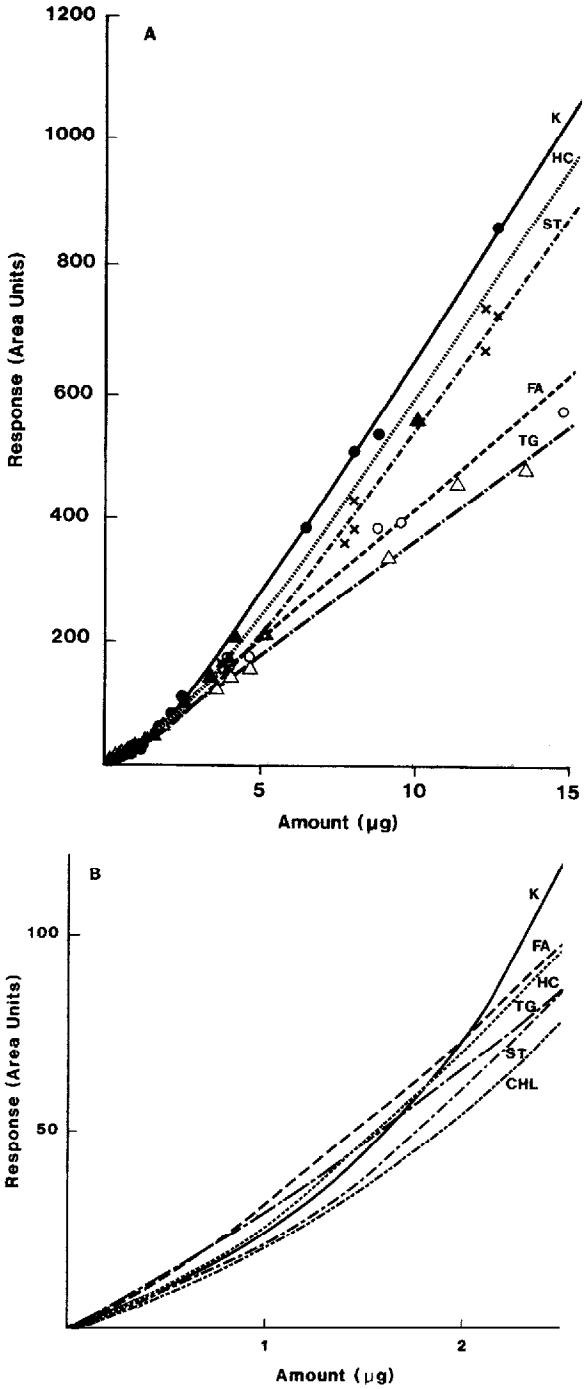

Fig. 1. Plot of peak areas (nominal units) *versus* amount of lipid applied to the rod. (A) At high loadings. ○ = FA (free fatty acids); ▲ = HC (hydrocarbons); ● = K (ketones); × = ST (sterols); △ = TG (triacylglycerols). (B) At low loadings (< 2 µg). CHL = chlorophyll. Data points in B omitted for clarity.

this feature in some detail since seawater analyses require accurate determinations of sub-microgram amounts for most lipid classes. Representative FID response curves for individual lipids are shown in Fig. 1. Each of the FID response curves is curvilinear although linear approximations can be made over small intervals. Linear fits to the data above about 3 μg do not extrapolate through zero-zero. The slopes of the curves are not in constant ratio and some curves cross. Relative responses at 3 different loadings are shown in Table I. These data are consistent with recent studies[10,11] and clearly show that correction factors can only be used at a specified loading[7] which undoubtedly accounts for some of the variations between correction factors reported by different researchers. Calibration curves, or polynomial fits to the data[13], are therefore essential when analysing samples containing mixtures of lipid classes spanning a wide range of concentrations.

Many TLC–FID studies use an internal standard in conjunction with correction factors for quantification of lipid concentrations. This procedure can only give approximate values since it assumes a linear FID response, and deviations from the true value are likely to be large at loadings less than 2–3 μg. The qualitative composition of many biological samples is likely to be known so the choice of an internal standard presents few problems. Also, sufficient material is often available which allows for multiple analyses both with and without the internal standard. This is not the case for most environmental marine samples where not only are lipids commonly in low abundance but the presence of unusual lipids or breakdown products often makes it difficult to choose a suitable internal standard. Parrish and Ackman[3] and Delmas *et al.*[13] suggest the use of the ethyl ketone hexadecan-3-one for seawater samples on the assumption that natural concentrations of ketones are low. However, as we show later, this is not true of some marine samples, particularly sediments, and also the response curves for alkyl ketones deviate significantly from linearity for amounts below 1 μg (Fig. 1). Nonetheless, the method is undoubtedly satisfactory for many seawater analyses[13].

In our work, we have adopted the approach of ensuring that the sensitivity of our instrument is kept constant so that peak areas can be directly converted to amounts using appropriate calibration curves. The sensitivity and reproducibility of the FID response is monitored using suitable external standards such as a long-chain alkane dissolved in hexane. To maintain sensitivity and acceptable baseline stability we found it necessary to periodically (every 3–4 weeks) disassemble and clean the

TABLE I

RELATIVE FID RESPONSES OF SELECTED LIPID CLASSES DETERMINED AT DIFFERENT LOADINGS

Lipid class	*Loading (μg)*		
	0.5	*2*	*10*
Triacylglycerols	1.0	1.0	1.0
Ketones	0.77	1.01	1.62
Sterols	0.75	0.96	1.39
Fatty acids	0.92	1.13	1.05
Hydrocarbons	0.78	0.98	1.44

FID, and care had to be taken that the FID collector was correctly aligned since this can markedly affect the sensitivity[15].

The main uncertainties associated with this external calibration technique are the errors in spotting the sample onto the rod. Values obtained using micropipettes were reproducible to within ±8% and slightly better reproducibility (±6%) was obtained using a 5-μl syringe. The latter was preferred when multiple spotting of a sample was required. Another difficulty, common to all TLC–FID analyses, is that FID responses of all compound classes are not known. Most of the peaks are complex mixtures of compounds and it may be unrealistic to assume that each will have a similar FID response to the standard compound chosen to be representative of that class. Also, the limited resolution achievable on the Chromarods inevitably results in poor separation of some compounds classes (*e.g.* wax esters and hydrocarbons, sterols and diacylglycerols, 4-methyl sterols and *n*-alkanols etc.), and these can have quite different FID responses. These problems can only be overcome by improvements to the design of the Iatroscan flame ionisation detector[15], perhaps combined with techniques such as copper sulphate pretreatment of the rods[12].

Lipid composition of seawater particulate matter

In 1978, Cauwet[27] remarked that very little was known about the organic composition of particulate matter in the oceans. This situation still prevails despite an increasing awareness of the importance of particulate matter in marine food chains and geochemical processes. Several studies have concentrated on total fatty acids and hydrocarbons (*e.g.* refs. 28–30), and there are reports of a few other important biochemicals such as sterols, fatty acid esters, alcohols and ketones (*e.g.* refs. 31–38). A common practice has been to saponify the lipid extract, so that little is known about the forms in which fatty acids exist in seawater (*i.e.* free, triacylglycerols, phospholipids etc.). Few studies have presented data for more than 3 lipid classes in the same water samples. This situation is gradually changing with the more widespread use of Iatroscan TLC–FID[3,13].

Fig. 2B shows a chromatogram of non-polar lipids isolated from particulate matter in surface waters from an oceanic station off the East Australian coast. These

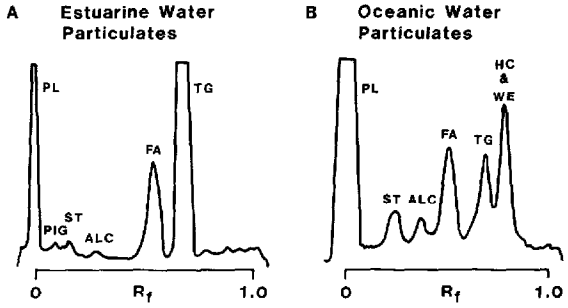

Fig. 2. TLC–FID chromatogram of non-polar lipids from seawater particulate matter, separated using the solvent system hexane–diethyl ether–acetic acid (60:17:0.15). Estuarine water (A) is from the Derwent Estuary, Hobart, Tasmania; oceanic water (B) is from the East Australian coast at 32°S 155°E. PL = polar lipids; PIG = unidentified pigments; ALC = alcohols; WE = wax esters; other abbreviations as in Fig. 1.

oligotrophic waters contain low concentrations of nutrients and hence a small standing stock of phytoplankton. Most of the organic matter consists of polar "lipids" (mainly pigments and degraded organic matter), but small amounts of sterols, alcohols, free fatty acids and triacylglycerols can be detected using the TLC–FID technique. Note that this chromatogram was obtained from 5% of the material extracted from 8 l of seawater, so in more productive coastal waters a few hundred ml of water would provide sufficient material to be analyzed using this method.

Quantitative data for this surface sample and four deeper samples are shown in Table II. Total lipid concentrations range from 3 to 10.7 μg/l, with the lowest value occurring at 120 m. These total values have an uncertainty of at least 20% since the FID response of the polar material which makes up the bulk of the sample is not known. We assumed a response similar to phospholipids, which is consistent with work by Delmas *et al.*[13], who found similar responses for chlorophyll, phospholipid and cholesterol. Our data also indicate very similar responses for chlorophyll *a* and cholesterol at low loadings (Fig. 1B). Our values for total lipids are similar to concentrations reported by Tanoue *et al.*[39] for other oceanic regions (3.3–11.5 μg/l), and as expected are much lower than those found in areas closer to shore such as in the Gulf of Mexico[31] (12–70 μg/l).

Particulate matter in seawater consists of both living cells (mainly phytoplankton and bacteria) and non-living detrital matter. During periods of high phytoplankton production, living cells may account for more than 80% of the particulate matter but in oligotrophic waters their proportion is expected to be considerably less. The lipid composition of the seawater particulate matter will depend on the number of living cells present, and it can therefore provide a measure of the relative proportion of living and non-living matter. Labile lipids such as triacylglycerols are rapidly degraded in seawater after cell lysis so high concentrations would not be expected when detrital matter greatly predominates. This point is illustrated by the two TLC–FID chromatograms in Fig. 2. A typical lipid distribution in seawater particulate matter from an estuary during a phytoplankton bloom is shown in Fig. 2A. Triacylglycerols are abundant, and the distribution is very similar to those of diatoms and other algae (Fig. 3). Microscopic examination of the sample revealed high concentrations of dinoflagellates, principally *Ceratium tripos*. By contrast, the TLC–FID chromato-

TABLE II

DEPTH PROFILE OF LIPIDS CONCENTRATIONS (μg/l) IN PARTICULATE MATTER FROM A STATION IN THE TASMAN SEA (32°S, 155°E)

Lipid class	*Depth (m)*				
	0	*30*	*60*	*90*	*120*
Triacylglycerols	0.84	0.50	0.60	0.60	0.30
Free fatty acids	1.0	1.5	1.2	1.1	0.47
Alcohols	0.10	0.02	TR*	0.03	0.05
Sterols	0.30	0.44	0.20	0.48	0.07
Other neutrals	0.40	0.07	0.35	3.1	0.17
Polar lipids	7.3	4.5	3.0	5.4	2.0

* TR = Trace amounts (<0.01 μg).

gram obtained from particulate matter in oligotrophic oceanic waters (Fig. 2B) is very different. The concentration of triacylglycerols is similar to that of free fatty acids, and the ratio of triacylglycerols to sterols is much smaller than the value for the estuarine sample. Such ratios which compare the lipid concentrations of a labile lipid with that of a more resistant lipid may possibly provide additional indices of phytoplankton biomass and physiological state to supplement estimates based on chlorophyll *a* concentrations.

Two previous studies[13,23] of lipid concentration in stratified water systems have used TLC–FID to demonstrate major changes in the amounts and proportions of various lipids with water depth. At the oceanic station described here the concentrations of major non-polar lipid classes show little variation within the top 90 m where the water is well mixed (temperature 20.21–20.24°C; salinity 35.622‰). However, concentration values at 120 m, which is below the thermocline are slightly lower which probably reflects the smaller population of phytoplankton there.

The concentrations of free sterols in particulate matter measured by TLC–FID at this station are slightly higher than values obtained using gas chromatography for intertropical Atlantic waters (0.01–0.07 μg/l)[41] but are within the range reported for dissolved plus particulate sterols in North Atlantic waters (0.12–1.3 μg/l)[35]. Considerably higher concentrations are found in coastal areas[36,37]. Further analysis of the sterol fractions of this oceanic sample by capillary gas chromatography (GC) showed that the distributions were very complex with cholesterol, 24-methylcholesta-5,22*E*-dien-3β-ol, 24-methylenecholesterol, 24-ethylcholesterol and 24-ethylcholesta-5,22*E*-dien-3β-ol as major components (*ca.* 75% of total sterols)[40]. Agreement between total sterol concentration data calculated by GC and by TLC–FID was generally quite good, but the TLC–FID values were generally 10–30% higher probably due to the presence of other compounds with a similar polarity to sterols such as diacylglycerols, which coeluted in the TLC–FID chromatogram. Better separations could be achieved using different solvent systems but at the expense of poor separation of less polar compounds. Double development techniques could be used to improve the quantitation but inter-rod variability made it difficult to choose an appropriate part on the rod to terminate the first pass throught the flame ionisation detector.

Surprisingly few data are available for lipid concentrations in oceanic areas and more research is needed before meaningful comparisons can be made between data sets. The problem of adequately covering such large areas of ocean surface still remains but the situation can only improve with the more widespread use of rapid and sensitive methods based on techniques such as TLC–FID.

Lipid compositions of marine organisms

The lipid compositions of marine organisms vary widely but usually a single solvent system is sufficient to separate non-polar lipids of interest. Representative TLC–FID chromatograms for selected marine organisms are shown in Fig. 3.

Thalassiosira eccentrica (Fig. 3A) is a common neritic diatom which reaches high concentrations in upwelling areas, such as those off Peru. In culture, this species produces large quantities of triacylglycerols and polar lipids, with comparitively small concentrations of sterols and hydrocarbons. Note that the triacylglycerol peak shows some tailing due to partial separation of triacylglycerols differing in degree of un-

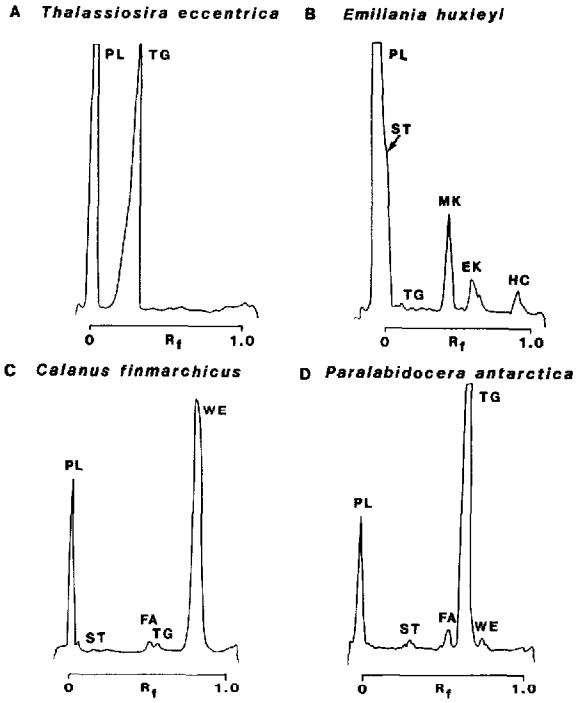

Fig. 3. TLC–FID chromatogram of non-polar lipids from two species of phytoplankton (A and B) separated using the solvent system hexane–diethyl ether (96:4) and Zooplankton (C and D) separated using the solvent system hexane–diethyl ether–acetic acid (60:17:0.15). MK = methyl ketones; EK = ethyl ketones; other abbreviations as in Figs. 1 and 2.

saturation. On silica S-II Chromarods polyunsaturated triacylglycerols are less mobile than those containing 0 to 3 double bonds. The TLC–FID technique is particularly suited for lipid analysis of cultured phytoplankton since the amount of cells harvested may be quite small. One useful application would be to study changes in the proportion of lipids as a culture ages and the cells change from exponential to stationary phase growth.

The coccolithophorid *Emiliania huxleyi* (Fig. 3B) is very widely distributed in the sea and can be a major contributor of organic matter found in marine sediments[25]. Although it is not usually the dominant phytoplankton, in some environments concentrations of up to 10^8 cells/l have been observed[42]. This alga has an unusual lipid composition which is dominated by very-long-chain (C_{37}–C_{39}) unsaturated methyl and ethyl ketones, methyl and ethyl esters of a 36:2 fatty acid and 37:3 and 38:3 *n*-alkenes (described in detail elsewhere[25,43]). These lipid classes are separated very well by the hexane–diethyl ether (96:4) solvent system, and thus the TLC–FID technique provides a ready means of detecting these, and other, unusual lipids in phytoplankton species. We have screened a wide range of algal species, but these unusual lipids have been detected only in a few algae of the class Prymnesiophyceae, implying that they may be good markers for organic matter derived from these algae. Note that the C_{37} methyl ketones have higher R_F values than medium-chain C_{17} and C_{19} methyl ketones chromatographed under the same conditions,

indicating that the length of the alkyl chain does influence retention behaviour. Also, nonadecan-3-one is difficult to resolve from the very-long-chain methyl ketones so this compound is not the most appropriate internal standard for samples likely to contain lipids from the Prymnesiophyceae.

The calanoid copepod *Calanus finmarchicus* is common in the northern waters around Britain. It is very lipid rich and the TLC–FID chromatogram shows that wax esters are far the major lipid present (Fig. 3C). The small amount of polar lipid is in marked contrast to the abundance of these lipids in the phytoplankton diet (contrast Fig. 3C with Fig. 3A which shows a typical diatom lipid distribution). Small amounts of sterols, free fatty acids and triacylglycerols were also detectable using TLC–FID.

The TLC–FID chromatogram of another calanoid copepod *Parlabidocera antarctica* which is found only in Antarctic waters, is shown in Fig. 3D. This sample of animals was obtained from a saline Antarctic lake and its lipid composition is unusual in that the major storage lipid is triacylglycerols and not wax esters as in *C. finmarchicus*. Earlier studies[44] suggested that copepods in polar regions synthesize mainly wax esters as an energy store but these lipids are very minor constituents in *P. antarctica*. This species may well synthesize more wax esters in its normal oceanic environment, since its diet there would be quite different, but this has yet to be tested. In other respects, the distributions of non-polar lipids in the two copepods are similar (Fig. 3C and D).

The high sensitivity of the TLC–FID technique lends itself to the analysis of small numbers of copepods, and in the case of lipid-rich species such as *C. finmarchicus* and *C. pacificus*[21], the lipid composition of a single animal can be determined. This should permit a range of biochemical experiments to be carried out comparing the influence of diet, sex and growth stage on the lipid composition of copepods and other small crustaceans.

Compositions of lipids in sediments

Sediments contain a diverse range of organic compounds derived from organisms in the sediment and from the overlaying water column. The lipid distributions are extensively modified by chemical and biological processes and are therefore typically more complex than the distributions found in seawater or marine organisms. There has been considerable progress in identifying many of the compounds present and in using this information to determine likely origins for the organic matter. However, the determination of the concentrations of lipid classes has been difficult and usually obtained from the summation of concentrations of individual compounds determined by GC or HPLC, rather than by a more direct and rapid method such as TLC–FID.

A TLC–FID chromatogram of lipids extracted from an oxic coral reef sediment is shown in Fig. 4. Non-polar lipids comprise only 8.1% of the lipid extract and consist of free fatty acids (5.9 μg/g), sterols (6.9 μg/g), wax esters and hydrocarbons (1.8 μg/g), alcohols (0.9 μg/g) and triacylglycerols (1.4 μg/g). Numerous other non-polar lipids are present in small amounts which cause an uneven baseline and limit the accuracy with which peak areas can be determined. In general, the concentrations in this sediment are intermediate between those found in deep-sea sediments and organic-rich sediments deposited in areas of high productivity. For example, a typical concentration of free sterols in organic-rich coastal sediments from Peru and

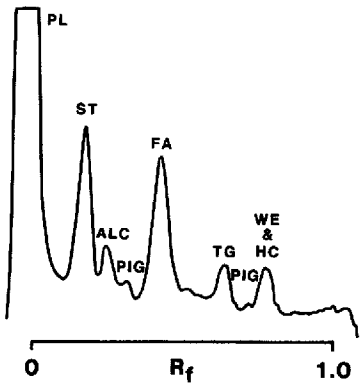

Fig. 4. TLC–FID chromatogram of non-polar lipids isolated from a Coral Sea reef surface sediment. The solvent system used was hexane–diethyl ether–acetic acid (60:17:0.15). Abbreviations as in Figs. 1 and 2.

Walvis Bay is about 100 μg/g[39,45], whereas in deep-sea[46] and intertidal[47] areas concentrations are usually less than 3 μg/g.

Significant qualitative and quantitative differences are found between the lipid distributions in sediments deposited under different conditions. Fig. 5 shows two sets of TLC–FID chromatograms obtained from organic-rich anoxic sediments at three depths in a core obtained from a saline Antarctic lake. Two solvent systems were used to separate all the lipid classes of interest. In the surface sediment, the major non-polar lipids are very long-chain unsaturated ketones and esters which are structurally similar to those produced by *E. huxleyi* (contrast Fig. 5 with Fig. 3B). These compounds are probably derived from a species of small flagellate found in the lake which has not yet been identified[23]. In the 65–70 cm section these compounds are much less abundant and at 125–130 cm they are barely detectable. Since such compounds are known to be very stable in sedimentary environments[25] these concentration data provide a record of the changes in water column populations of the source algae throughout the history of the lake.

Significant amounts of sterols and long-chain alcohols are present in each of the three core sections but, in contrast with the coral reef sediment, free fatty acids are not abundant and triacylglycerols could not be detected. TLC–FID analyses of particulate matter in the lake showed that high concentrations of fatty acids and triacylglycerols were present[23], so clearly these labile lipids have been extensively degraded by bacteria in the sediment, resulting in a relative enhancement in abundance of the resistant sterols and long-chain ketones.

Alcohols are also readily degraded in sediments, so it is likely that in the Ace Lake sediments they are derived from the sediment bacteria and not from organisms in the water column.

Sterol concentrations in the 0–5, 65–70 and 125–130 cm sections were calculated to be 370, 790 and 539 μg/g (dry wt. sediment) from the TlC–FID response. Comparable values were determined by summation of GC–FID peak areas. These concentrations are more than an order of magnitude higher than those found in most sediments and indicate high algal productivity throughout the history of the lake.

Most sediments contain high concentrations of chlorophylls, carotenoid pig-

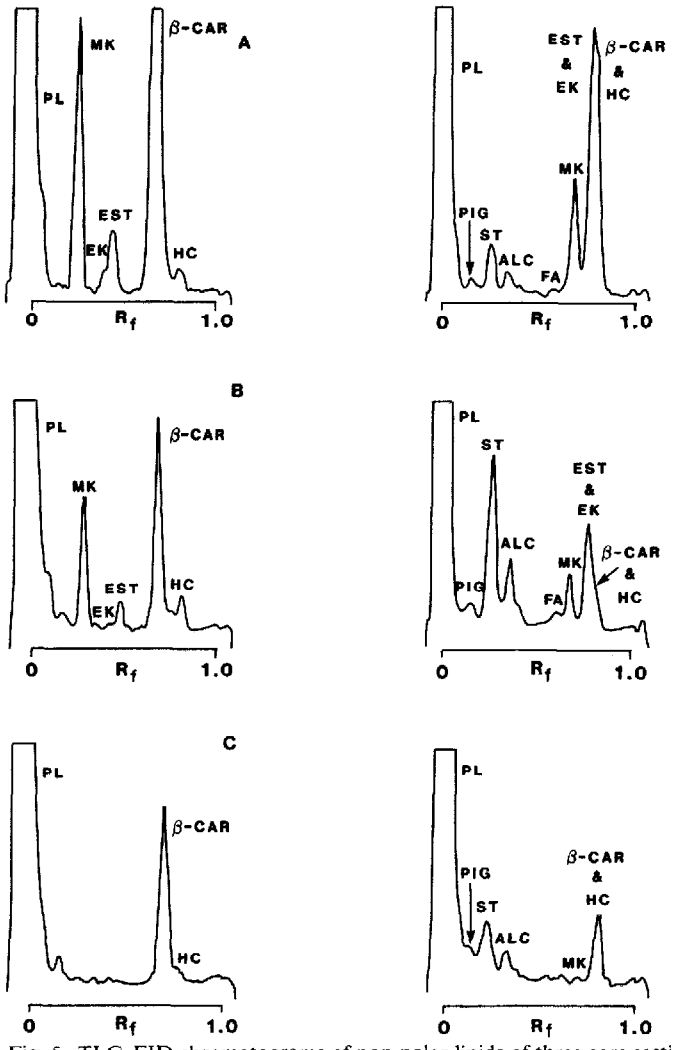

Fig. 5. TLC–FID chromatograms of non-polar lipids of three core sections from Ace Lake, an Antarctic saline lake. (A) 0–5 cm; (B) 65–70 cm; (C) 129–130 cm. Two developing solvent systems were used: left, hexane–diethyl ether (96:4); right, hexane–diethyl ether–acetic acid (60:17:0.15). β-CAR = β-Carotene; other abbreviations as in Fig. 1–3.

ments and their degradation products which can severely complicate TLC–FID analyses. Carotenoid pigments are abundant in the Antarctic lake sediments, and β-carotene appears as a major peak chromatographing just before *n*-alkanes (Fig. 5). The peak area varied greatly in different analyses of the same sample, indicating (not surprisingly) that TLC–FID is not the method of choice for pigment analysis. Pigments can usually be seen on the rod after development and thus peaks in the TLC–FID chromatogram due to pigments can be readily assigned.

Most sediment and seawater samples contain a high proportion of polar lipids which present several practical problems. It is often difficult to spot samples onto the

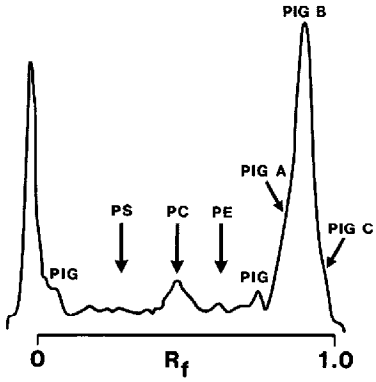

Fig. 6. TLC–FID chromatogram of polar lipids from surface sediment from Ace Lake, an Antarctic saline lake. The developing solvent system used was chloroform–methanol–water (80:15:2). PIG = Unidentified pigment; PS = phosphatidylserine; PC = phosphatidylcholine; PE = phosphatidylethanolamine. Pigments A, B and C have the same retention time as fucoxanthin, chlorophyll *a* and chlorophyll *c*, respectively.

rods since 100 μg or more of the sample has to be applied to permit identification of non-polar lipids, which comprise only a few percent of the sample. This polar material is poorly soluble in hydrocarbon solvents requiring polar solvent mixtures, such as chloroform–methanol, to completely dissolve the sample. Alternatively, if information on the polar lipids is not required then the polar lipids can be removed on a short column of silicic acid, or less polar solvent mixtures such as hexane–isopropanol can be used for extraction[48].

The use of polar solvent systems for TLC development of the polar lipids in the Ace Lake 0–5 cm sediment (Fig. 6) indicated that most of this material was more mobile than phosphatidylethanolamine and had similar retention behaviour to pigments such as chlorophylls *a* and *c* and fucoxanthin. Visual inspection of the rods showed that most of the peaks in the TLC–FID chromatogram corresponded to pigmented material and it seems that the TLC–FID method will be difficult to apply to analyses of phospholipids or other polar lipids in sediments unless detectors specific for phosphorus become available.

CONCLUSIONS

TLC–FID has been shown to be a very valuable technique for the analysis of non-polar lipids in samples from the marine environment including phytoplankton, marine animals, seawater particulate matter and sediments. [This technique is not as easy to apply as it appears at first sight and precisions better than 10% are difficult to achieve.] The curvilinear response of the detector, and major differences in the detector response of compounds of interest still present difficulties, making the use of FID calibration curves essential for good results. However, the method is much more rapid and no less accurate than gravimetric techniques. This situation should be assisted by improvements to detector design and sample application techniques. The high concentrations of pigments and degraded organic matter in sediments and

seawater present some practical problems but the benefits of rapid screening of samples combined with high sensitivity and the ability to resolve and quantify most non-polar lipids in one or two analyses should result in more widespread use of this technique.

ACKNOWLEDGEMENTS

We thank Mr. H. R. Burton, Dr. S. W. Jeffrey, Dr. E. D. S. Corner and Dr. J. R. Sargent for providing samples, Mr. P. L. Stevenson for valuable technical assistance, and Professor R. G. Ackman as reviewer for helpful suggestions.

REFERENCES

1 M. W. Rigler, R. L. Leffert and J. S. Patton, *J. Chromatogr.*, 277 (1983) 321.
2 M. Tanaka, K. Takase, J. Ishii, T. Itoh and H. Kaneko, *J. Chromatogr.*, 284 (1984) 433.
3 C. C. Parrish and R. G. Ackman, *J. Chromatogr.*, 262 (1983) 103.
4 D. Vandamme, V. Blaton and H. Peeters, *J. Chromatogr.*, 145 (1978) 151.
5 T. Itoh, M. Tanaka and H. Kaneka, in J. C. Touchstone and D. Rogers (Editors), *Thin Layer Chromatography: Quantitative Environmental and Clinical Applications*, Wiley , New York, 1980, p. 536.
6 R. T. Crane, S. C. Goheen, E. C. Larkin and R. Ananda, *Lipids*, 18 (1983) 74.
7 J. K. Kaitaranta and N. Nicholaides, *J. Chromatogr.*, 205 (1981) 339.
8 Y. Takemoto, *Kawasaki Med. J.*, 6 (1980) 1.
9 M. T. Clandinin, M. Foot and L. Robson, *Comp. Biochem. Physiol.*, 76B (1983) 335.
10 C. C. Parrish and R. G. Ackman, *Lipids*, 20 (1985) 521.
11 M. L. Selucky, *Lipids*, 20 (1985) 546.
12 T. N. B. Kaimal and N. C. Shantha, *J. Chromatogr.*, 288 (1984) 177.
13 R. P. Delmas, C. C. Parrish and R. C. Ackman, *Anal. Chem.*, 56 (1984) 1272.
14 E. R. Farnworth, B. K. Thompson and J. K. G. Kramer, *J. Chromatogr.*, 240 (1982) 463.
15 H. Read, *Lipids*, 20 (1985) 510.
16 D. M. Bradley, C. R. Rickards and N. S. T. Thomas, *Clin. Chim. Acta*, 92 (1979) 293.
17 M. Ranný, M. Zbirovský, M. Bláhová, V. Růžička and Š. Truchlik, *J. Chromatogr.*, 247 (1982) 327.
18 J. K. Kaitaranta and R. G. Ackman, *Comp. Biochem. Physiol.*, 69B (1981) 725.
19 S. M. Innis and M. T. Clandinin, *J. Chromatogr.*, 205 (1981) 490.
20 R. B. Kellogg and J. S. Patton, *Mar. Biol.*, 75 (1983) 137.
21 J. L. Hakanson, *Limnol. Oceanogr.*, 29 (1984) 794.
22 G. van Graas, T. C. Viets, J. W. de Leeuw and P. A. Schenck, in M. Bjorøy, P. Albrecht, C. Cornford, K. de Groot, G. Eglinton, E. Galimov, D. Leythaeuser, R. Pelet, J. Rullkötter and G. Speers (Editors), *Advances in Organic Geochemistry, 1981*, Wiley, New York, 1983, p. 471.
23 J. K. Volkman, H. R. Burton, D. A. Everitt and D. I. Allen, *Develop. Hydrobiol.*, in press.
24 H. R. Harvey, M. D. Richardson and J. S. Patton, *Deep-Sea Res.*, 31 (1984) 403.
25 J. K. Volkman, G. Eglinton, E. D. S. Corner and J. R. Sargent, in A. G. Douglas and J. R. Maxwell (Editors), *Advances in Organic Geochemistry, 1979*, Pergamon Press, 1980, p. 219.
26 H. R. Harvey and J. S. Patton, *Anal. Biochem.*, 116 (1981) 312.
27 G. Cauwet, *Oceanol. Acta*, 1 (1978) 99.
28 P. G. Desideri, L. Lepri, D. Heimler, S. Giannessi and L. Checchini, *J. Chromatogr.*, 284 (1984) 167.
29 M. Goutx and A. Saliot, *Mar. Chem.*, 8 (1980) 299.
30 E. Tanoue and N. Handa, *Trans. Tokyo Univ. Fisheries*, 5 (1982) 85.
31 N. C. Kennicut II and L. M. Jeffrey, *Mar. Chem.*, 10 (1981) 389.
32 A. Saliot, M. Goutx, A. Fevrier, D. Tusseau and C. Andrie, *Mar. Chem.*, 11 (1982) 257.
33 A. Saliot, C. Andrie, M. Goutx, A. Fevrier, D. Tusseau and J. C. Marty, *Mar. Chem.*, 12 (1983) 236.
34 R. B. Gagosian, J. K. Volkman and G. Nigrelli, in M. Bjorøy, P. Albrecht, C. Cornford, K. de Groot, G. Eglinton, E. Galimov, D. Leythaeuser, R. Pelet, J. Rullkötter and G. Speers (Editors), *Advances in Organic Geochemistry, 1981*, Wiley, New York, 1983, p. 369.
35 R. B. Gagosian, *Geochim. Cosmochim. Acta*, 39 (1975) 1443.
36 A. Saliot and M. Barbier, *Deep-Sea Res.*, 20 (1973) 1077.

37 A. Kanazawa and S. Teshima, *J. Oceanogr. Soc. Japan*, 27 (1971) 207.
38 M. Ehrhardt, Ch. Osterroht and G. Petrick, *Mar. Chem.*, 10 (1980) 67.
39 E. Tanoue, N. Handa and M. Kato, *Trans. Tokyo Univ. Fisheries*, 5 (1982) 65.
40 J. K. Volkman, *Org. Geochem.*, in press.
41 A. Saliot and D. Tusseau, *Org. Geochem.*, 7 (1984) 53.
42 G. Berge, *Sarsia*, 6 (1962) 27.
43 J. K. Volkman, G. Eglinton, E. D. S. Corner and T. E. V. Forsberg, *Phytochemistry*, 19 (1980) 2619.
44 R. F. Lee and J. Hirota, *Limnol. Oceanogr.*, 18 (1973) 227.
45 A. M. K. Wardroper, J. R. Maxwell and R. J. Morris, *Steroids*, 32 (1978) 203.
46 C. Lee, J. W. Farrington and R. B. Gagosian, *Geochim. Cosmochim. Acta*, 43 (1979) 35.
47 J. K. Volkman, F. T. Gillan, R. B. Johns and G. Eglinton, *Geochim. Cosmochim. Acta*, 45 (1981) 1817.
48 N. S. Radin, in J. M. Lowenstein (Editor), *Advances in Enzymology, Vol. 72, Part D, Lipids*, Academic Press, New York, 1981, p. 5.