Calibration of the Iatroscan-Chromarod System for Marine Lipid Class Analyses

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ABSTRACT

A two-step development procedure with partial scanning after the first development resolves seawater lipids into seven classes. The low concentration in seawater of some of these classes necessitates calibration close to the detection limit of the flame ionization detector (FID). From 0.2 to 5.0 μg the FID response usually is curvilinear, necessitating multilevel calibration. Interrod precision was poor for most of this range, and this prompted an investigation of factors affecting FID responses.

Peak areas were found to depend on the distribution of lipid material on the Chromarod. Also investigated were the effects of temperature, humidity, acid cleaning and double developments. All of these factors had some effect on R_f values, on absolute FID responses and on reproducibility, but they were not the major cause of variability in the Iatroscan-Chromarod system. Similarly, double developments improve peak shape and response, but result in only a small increase in precision for some compounds. The differences among the FID responses obtained from 10 rods within a set imply that the normalization of FID responses to that of an internal standard, or the use of intrarod rather than interrod data, should result in an increase in reproducibility. Neither of these approaches was found to improve the precision for all lipid classes. An understanding of both interrod and intrarod variability, and the use of calibration curves, is essential for accurate determination of the lipid classes of 1 liter of seawater.

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INTRODUCTION

In contemporary oceanography, there are few other techniques currently available which can compare with TLC/FID for the number of marine organic classes that can be measured, the number of samples that can be processed, or for the simplicity and price of equipment required.

The Iatroscan-Chromarod system would appear to be well suited for an examination of marine organic classes in an oceanographic context. Thin-layer separations on the reusable Chromarods are both excellent and versatile (1, 2), the Iatroscan flame ionization detector (FID) system is robust enough to function well at sea (3), and the scanning time is very rapid: a chromatogram is obtained in less than a minute. However, the relationship between FID response and compound load is not straightforward (3-5), and it has been suggested that the Iatroscan has limited applicability in the quantitative analysis of lipids (1,4).

The present study is a detailed examination of the magnitude and precision of FID responses of lipids used as representatives of marine classes separated on Chromarods.

MATERIALS AND METHODS

Apparatus and Operating Conditions

All separations were performed on silica-coated Chromarods-SII. Shipboard FID scans (Fig. 1) were performed on an Iatroscan (Iatron Laboratories, Tokyo) Mark II analyzer which had the analogue and integral outputs connected to a two-pen Fisher Recordall (3). Land-based studies on FID response and precision were performed on an Iatroscan Mark III connected, via the analogue output, to a Spectra-Physics SP4200 computing integrator. Both Iatroscans were fitted with a push-button switch (normally closed) placed in a series with the microswitch (S_1) used to stop scans. With this arrangement any Chromarod FID scan could be interrupted at any time. The Iatroscans were operated with a hydrogen flow of 160 ml/min, a scan speed of 3.1 mm/sec and an air flow of 2000 ml/min.

Standards (Table 1) were dissolved together in chloroform or (in some cases) singly in isooctane. Standard solutions were spotted onto Chromarods with disposable 1 μl pipettes (Drummond Scientific Co., Broomall, Pennsylvania). All solvents used were 'distilled in glass' grade or better.

Experimental Procedure

Lipids were applied to flame-activated Chromarods and focused twice to the origin in acetone (3). The rods were deactivated for 10 min over a saturated solution of NaCl, and then equilibrated with the vapor of the first developing solvent system for 5 min before development. The lipids were separated in a stepwise sequence

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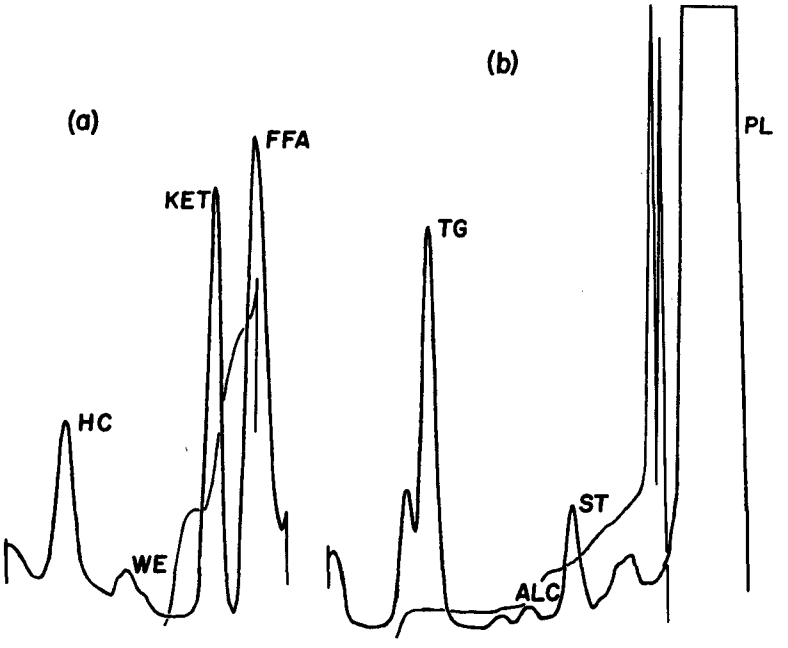

FIG. 1. Shipboard analysis of dissolved lipids in Scotian Shelf waters; abbreviations explained in Table 1. (a) 45-min development in hexane/diethyl ether/formic acid (98:2:0.2); partial scan. (b) Second development on the same rod: 45 min in hexane/diethyl ether/formic acid (80:20:0.2); full scan. Developing direction is from right to left, scanning direction is from left to right.

TABLE 1

Seawater Lipid Classes and Standards Used for their Identification and Calibration in the Iatroscan

Class	Abbreviation	Standards and Suppliers
Aliphatic hydrocarbon	HC	Nonadecane (Polyscience)
Wax and sterol esters	WE	Hexadecyl palmitate (Analabs)
Ethyl ketone	KET	Hexadecan-3-one[a] (K & K Labs)
Free fatty acid	FFA	Palmitic acid (Supelco)
Triglyceride	TG	Tripalmitin (Supelco)
Free alcohol	ALC	Hexadecan-1-ol (Polyscience)
Free sterol	ST	Cholesterol (Supelco)
Polar lipid	PL	Dihexadecanoyl lecithin (Supelco)

[a]Internal standard.

(2). After each day of use the rods were acid cleaned (5) and stored over a saturated solution of NaCl. Shipboard seawater analyses (Fig. 1) were performed on dichloromethane extracts of 1 liter aliquots of filtered water (3).

RESULTS AND DISCUSSION

Separations

A two-step development strategy was used to perform shipboard analyses of 'dissolved' lipid classes in filtered seawater (Fig. 1). The first solvent system resolves classes in the polarity range HC to FFA [Fig. 1(a): abbreviations are explained in Table 1]. The rod scan is stopped, using the push-button switch, at the lowest point on the tail of the FFA peak, and the remaining unpyrolyzed classes are then developed with a more polar solvent system [Fig. 1(b)]. This strategy resolves seven seawater lipid classes which have been identified by R_f values and by cospotting (Table 1).

Calibration Curves

For practical reasons it is necessary to reduce seawater sample size as much as possible when

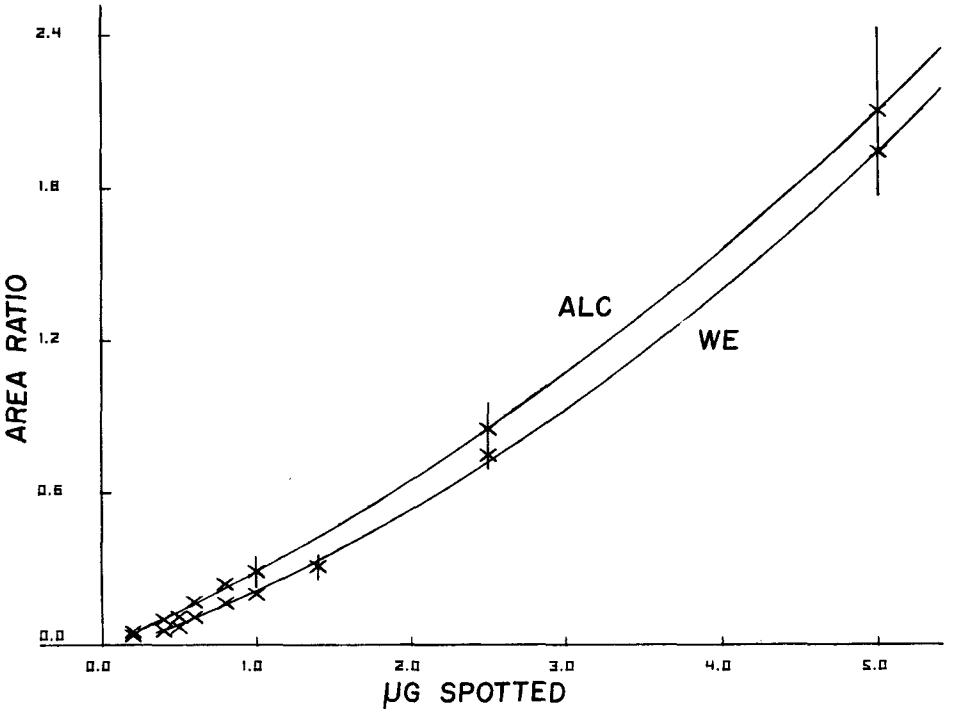

FIG. 2. Calibration curves. Computer-drawn quadratic regressions (Table 2) through response data from hexadecanol and hexadecyl palmitate taken in ratio to 3 μg of hexadecanone; error bars extend 1 standard deviation from the mean.

performing shipboard analyses. This means that the FID detector in the Iatroscan has to be operated quite near its detection limit for most lipid classes in most seawater samples, and detailed low-load calibration curves are necessary for the minor seawater lipid classes.

To investigate the linearity and precision of the FID response at low μg-loads, a single set of 10 Chromarods was used. On average, eight different loads were applied to the Chromarods, and about half of these loads were below 1 μg (Figs. 2 and 3). Coefficients of determination (r^2) were calculated for five different regression models (Table 2). In order to obtain an estimate of the precision of the analyses over this range, the coefficient of variation (CV) was calculated at each level, and it is the mean over the range 0.2 to 5.0 that is given in Table 2. The CV is the standard deviation expressed as a percentage of the mean, and in Table 2 six analyses were made, on average, with each μg-load.

Calibration curves in the range 0.2 to 5.0 μg are distinctly curvilinear (Figs. 2 and 3), and are best described using quadratic equations (Table 2). There is, however, only a small difference in the r^2 values for quadratic and linear regressions, but the latter pass through the x axis between 0.2 and 0.5 μg and are thus unsuitable for calibration at low loads.

A positive intercept on the load axis from a linear regression (6) will automatically result in an increase in response per μg with increasing load (4), because the equation describing the response contains a negative term. Any positive curvature [Table 2, (7-9)] will further aggravate this situation. Thus, single-level calibrations (10,11) should be used only when similar loads of samples and standards are scanned on Chromarods. When a range of lipid loads is to be analyzed, a multilevel calibration (Figs. 2 and 3) will produce more accurate results.

There also is little difference between the correlations for quadratic and power law regressions (Table 2). Power law calibrations have been applied to lipids scanned on boric acid impregnated Chromarods (12). The good fit obtained with power curves suggests similarities with nonlinear FID responses obtained from lipids after liquid chromatographic separations (13) or else after gas chromatographic separations (14).

By taking logarithms of both sides of a power

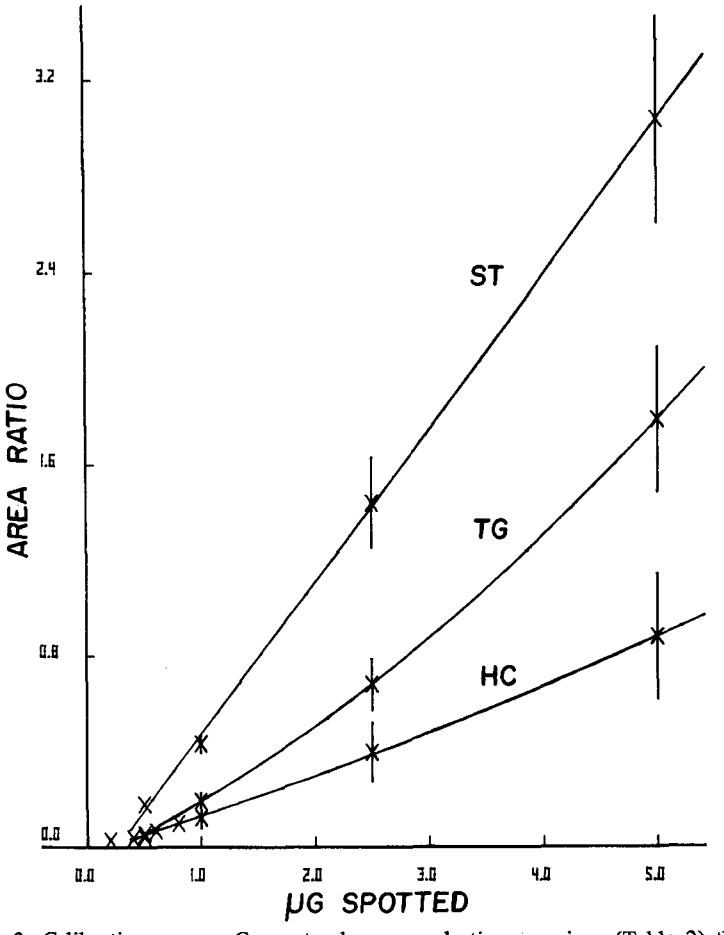

FIG. 3. Calibration curves. Computer-drawn quadratic regressions (Table 2) through response data normalized to responses from 3 μg of hexadecanone; error bars extend 1 standard deviation from the mean.

TABLE 2

Iatroscan FID Responses: Precision and Regressions at Low Loads (0.2 - 5.0 μg)

		r^2 Values					Quadratic constants		
Class	Mean CV	ae^{bx}	ax^b	a+b1nx	a+bx	$a+bx+cx^2$	a	b	c
HC	23	.874	.975	.805	.977	.999	.023	.15	.007
WE	17	.865	.984	.725	.985	.999	.030	.21	.038
TG	16	.883	.996	.864	.993	1.000	.067	.23	.029
ALC	19	.866	.995	.789	.993	1.000	.101	.27	.031
ST	11	.894	.998	.909	1.000	1.000	.164	.63	.002

law equation [1], the equation for a straight line is obtained [2]:

$$y = ax^b \quad [1]$$

$$\log y = \log a + b(\log x) \quad [2]$$

With Iatroscan data y, the dependent variable, is the measured peak area; x, the independent variable, is the load applied to the Chromarod; and log a, and b are constants; the y-intercept and slope of the line in equation [2]. If b = 1, the FID can be said to respond linearly (14).

Linear calibration curves have been obtained previously from Iatroscan data plotted on full

logarithmic graph paper (15,16). This suggests further support for the applicability of a general power law model to describe Iatroscan FID response.

Reproducibility

In the range 0.2 to 5.0 μg there is a general gradation in the standard deviation of the mean FID responses (Figs. 2 and 3). As noted previously (7), lower loads have lower standard deviations as do compounds with lower FID responses. The trend for the CV, however, is usually the opposite (15,17). Thus, the mean CV over a range that includes several data points at low loads is usually high (Table 2). Standard deviations significantly higher than 10% of the mean have been reported previously for low μg-loads (3,7,15,17).

The increase in standard deviation with increasing load suggests that simple linear regressions through the logarithms of the calibration data would be more accurate than nonlinear regressions through the untransformed data. This would not be the case if weighting factors were used with the nonlinear regressions. This argument has been applied to TLC-densitometry calibration data, which also is nonlinear (18). However, it should be noted that although the use of log-transformed data produces linear Iatroscan calibration curves (15,16), it does not obviate the necessity of performing multilevel calibrations.

The generally poor CV obtained for low loads with most of the representatives of the classes that usually are found as minor components in the lipids of seawater (Fig. 1; Table 2) prompted some detailed investigations into factors affecting FID responses.

Modification of FID Responses

A rapid method for collecting a large amount of FID data is to spot the same compound at various places on each of a set of 10 rods and to scan these rods without having developed the spots (4,5). Using this approach, a relationship between integrator peaks and solvents used in standard solutions was established. Hexadecanone spotted in 1 μl of isooctane gives tall, thin peaks whose area depends on the orientation of the Chromarod with respect to the FID burner. With rod orientation reversed, so that the side of the rod that was spotted passes nearest the top of the FID burner, the FID response was significantly higher. Rod orientation had no effect on hexadecanone spotted in chloroform. However, the peaks produced using chloroform as a spotting solvent were often broad and frequently were split near the top. The same situation occurs with tripalmitin spotted in chloroform/methanol 2:1 (5). The differences in peak shape resulting from spotting in the isooctane solution or the chloroform-based solutions are likely to be related to differences in solvent polarity and the ability of the solvent to move the material away from the point of application. With hexadecanone spotted in isooctane the lipid material must remain not only at the point of application in terms of displacement along the rod, but also in terms of displacement around the rod. Since there rarely is any sign of a peak on reburning the rod in any orientation, it appears that the distribution of material influences the proportion of ions registered by the integrator after being detected by the FID.

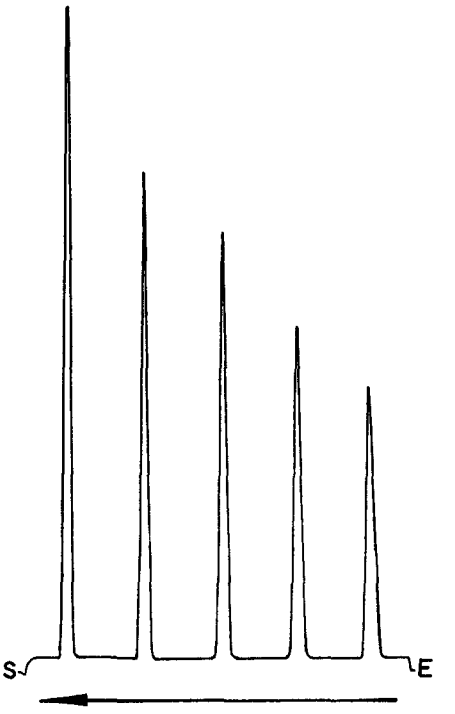

FIG. 4. Hexadecanone spotted in 1 μl of isooctane at 5 separate places on a Chromarod. The rod was developed, from right to left in the figure, for 40 min in 100% hexane; S and E denote the start and end of the FID scan.

It was anticipated that more regular peak shapes would be obtained from a rod that has been spotted at several places if the lipid material was developed. In order to avoid running separate peaks together it is necessary to use a solvent in which the lipid material is only slightly mobile (Fig. 4). This development almost invariably eliminates the appearance of split peaks in chromatograms; however, it causes a gradation in peak shapes along the rod (Fig. 4).

The lipid material which has spent the longest period of time in the developing solvent has the broadest-based peak. This corresponds to the familiar band spreading found in other chromatographic techniques. However, what is unexpected about these peaks is that there is a concomitant gradation in peak area along the rod: the broadest-based peaks have a lower area than the rest. This applies to both hexadecanone and tripalmitin (5). These observations, coupled with those from undeveloped spots, suggest that there is a relationship between the distribution of material on the Chromarod, the shape of the peaks produced on scanning, and the final area that is integrated.

If hexadecanone and tripalmitin are spotted together and developed in hexane/diethyl ether (80:20) they migrate to the same place on the Chromarod. The total peak area, however, is significantly larger than the sum of the areas of each compound when developed singly to the same place. This suggests that the increase in peak area per μg with increasing load (Figs. 2 and 3) does not depend directly on the nature of the material being pyrolyzed. It seems likely that the FID responds nonlinearly to the number of ions produced per unit area of Chromarod; or, for a given scan speed, it responds curvilinearly to the flow of ions per unit time. This argument may help explain why some compounds have a higher response at higher scan speeds (4). It also could help explain the differences in peak area observed with different band-widths of the same amount of material (Fig. 4). The FID passes through a complete range of responses as a band of material is burnt on a Chromarod. With a narrow band the FID will respond at a proportionally higher level on average than with a broad band, even if identical amounts of material are burnt in both cases. Thus the narrower band automatically will give a larger peak area.

Factors Affecting Silica Gel-Lipid Interactions

The indication of an apparent association between peak area and the distribution of lipid material on the silica suggested that an improvement in the reproducibility of the migration of a compound could lead to an improvement in the precision of the quantification of that compound.

It has long been known that the reproducibility of R_f values on TLC plates can be improved by controlling the relative humidity of the atmosphere in contact with the plate prior to development (19). A 10-min exposure of Chromarods to a constant relative humidity (between 30 and 75%, depending on the saturated salt solution used) does improve the reproducibility of R_f values when there are large

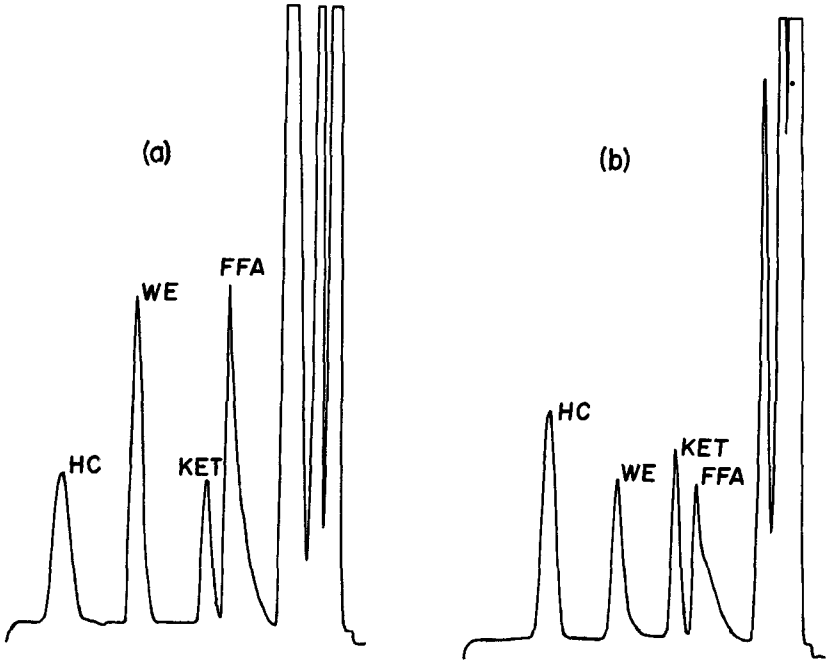

FIG. 5. Complete scans after 40-min developments, at (a) 24 C and (b) 3 C, of the same standard solution on the same rod in hexane/diethyl ether/formic acid (97.9:2:0.1).

changes in the relative humidity in the laboratory. However, it was found that on a run to run basis the improvement in the reproducibility of R_f values and the improvement in the precision of quantification were not statistically significant.

Similar conclusions can be drawn from other factors which might be considered to affect R_f values or FID responses. Developments performed at very different temperatures result in different R_f values and different FID responses (Fig. 5); however, the use of a constant temperature bath to maintain the developing temperature within a 0.3 C range did not result in a significant increase in precision.

After several developments on a set of Chromarods the R_f values and FID responses differ from those obtained from rods that have just been acid cleaned. However, it was again not possible to demonstrate an improvement in the standard deviation of the mean when rods were used for a maximum of only two development sequences before being acid cleaned.

These observations imply that, although a strict control of factors which can affect the migration of lipids does appear to improve the reproducibility of FID responses, the factors that were investigated are not the main cause of rod to rod or run to run variability in Iatroscan data.

Double Developments

The effect of developing solvents on recorder peak shapes (Fig. 4) suggested an investigation into the effect of double developments on FID responses and their reproducibility. A direct parallel to the gradation of peak shapes and areas (Fig. 4) can be found in chromatograms of neutral lipid standards [Figs. 6(a) and 7(a)]. HC, the most mobile class, gives the broadest-based peaks [Fig. 6(a)] and also the lowest FID response (Fig. 3). By producing a narrower peak it should be possible to increase the peak area [Fig. 4, (5)]. For HC this can be achieved by performing a second development, in the same solvent system, for a shorter length of time. This procedure does, on average, increase the peak area [Fig. 6(b)]. A double development of the TG peak produces similar results [Fig. 7(b)]. This time, however, two different solvent systems were used. The three remaining neutral lipid standards were moved from the point of application with a 40-min development in hexane/diethyl ether (80:20). After drying, the rods were redeveloped in hexane/diethyl ether/formic acid (98:2:0.1) for 30 min and then subjected to a partial scan to just after the ST peak [Fig. 7(b)].

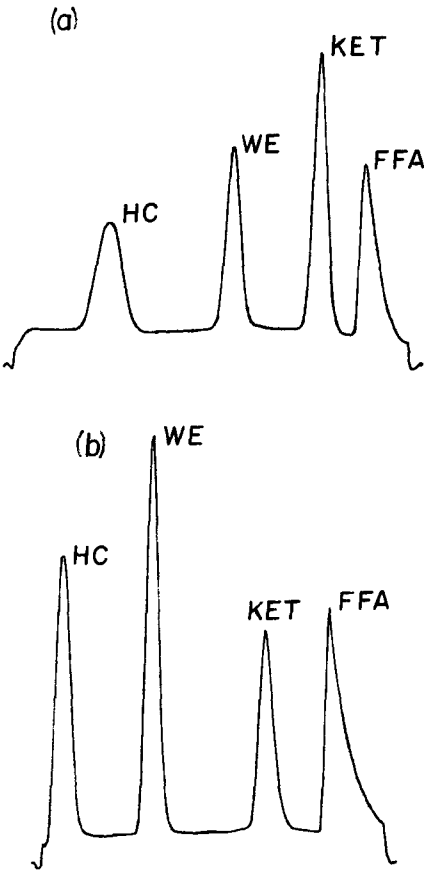

FIG. 6. Partial scans of 3 μg of each compound on the same rod; (a) after a 40-min development in hexane/diethyl ether/formic acid (98:2:0.1), and (b) after a double development (40 followed by 30 min) in the same solvent system.

In most neutral lipid separations, PL remains at the point of application and gives a comparatively large peak area considering its high proportion of oxygen [Fig. 7(a)]. Since PL is immobile in the solvent systems used it would seem that this is analogous to the situation described above for undeveloped rods, and thus the shape of the PL peak is almost entirely dependent on the polarity of the spotting solvent. If this analogy is correct, development of PL away from the point of application should reduce the peak area. This is indeed the case [Fig. 7(b)]. The phospholipid standard was moved from the origin with two 5-min developments in chloroform/methanol/water (50:40:10). After drying, the rods were subjected to a full scan. The chart speed on the computing integrator was routinely set at a slower rate for this scan and for reburns done immediately after it (Fig. 7), in order to save paper.

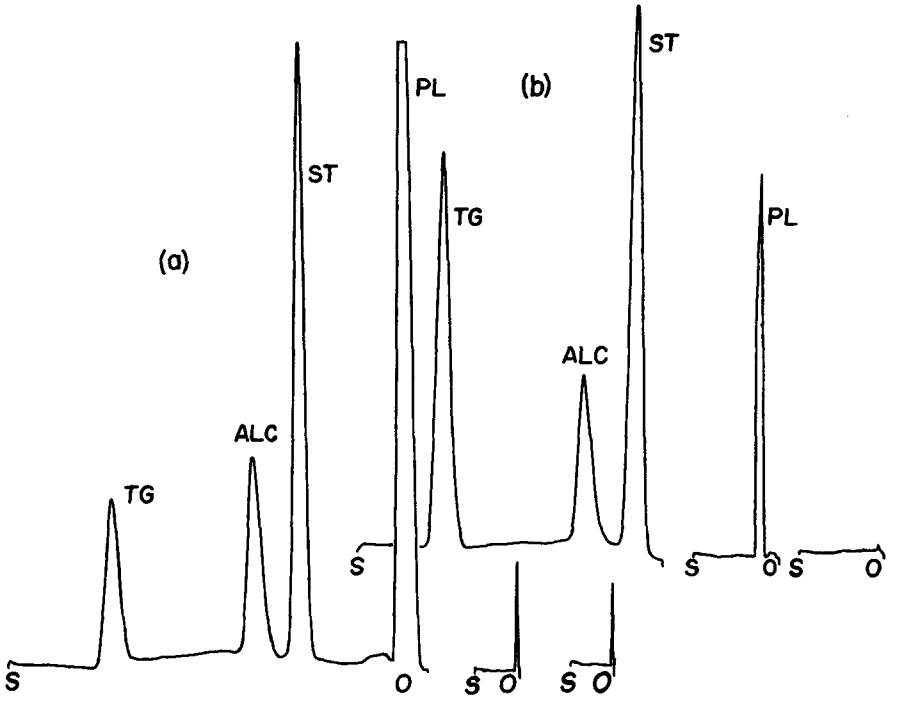

FIG. 7. Developments, scans and reburns after the partial scans in Fig. 6; attenuation is the same for all chromatograms, chart speed is slower for second scans and for reburns; 0 is the point of application, S denotes the starting point of a scan. (a) Complete scan after development for 40 min in hexane/diethyl ether (80:20), followed by two reburns (bottom center) at a slower chart speed. (b) Partial scan of neutral lipids after a double development in hexane-based solvent systems, followed by a complete scan at a slower chart speed after a double development in a chloroform-based solvent system, followed by a reburn (far right).

A further difference between developed and undeveloped PL peaks is the response on reburning the Chromarod (Fig. 7). Developed PL peaks rarely show as much as 1% of the original peak remaining on a reburn [Fig. 7(b)]. A reburn of undeveloped peaks, however, often results in a residual peak which is larger than 1% of the original area [Fig. 7(a)]. It is unlikely that a major proportion of this residual peak consists of the lecithin that was spotted. This material is immobile in phospholipid solvents, and the peak is not reduced by a further 95 to 99% on reburning [third scan of the rod at the point of application: Fig. 7(a)]. In fact, it takes more than 10 scans or else strong acid cleaning to reduce the peak to an undetectable level. The fact that development of the PL peak in a very polar phospholipid solvent alleviated the problem of peaks appearing on recombustion [Fig. 7(b)] implies that residue peaks on Chromarods can be an artifact of the distribution of material on the Chromarod. Some of these observations are similar to those suggested to be the result of the inclusion of phytin in polar lipid fractions scanned on Chromarods (20).

The ease with which FID responses can be altered by different lengths of exposure to solvent systems (Figs. 4, 6 and 7) implies that it would be difficult to make comparisons between FID responses in the literature (4) unless developments were performed for the same length of time in the same solvents. Because of the nature of calibration curves (Figs. 2 and 3) it also would be necessary to compare FID response ratios with the same loads: the variation in response ratios with varying loads has been discussed previously (7,15). Furthermore, rod conditioning can be expected to play some part in determining response ratios, and this also would have to be standardized before successful comparisons could be made. Nonetheless, if developing conditions within a laboratory are standardized and if calibration curves are constructed, a reasonably accurate assessment of the lipid class content of a sample should be obtained. A worthwhile exercise would be to test this by performing an interlaboratory calibration.

Since different Chromarods elicit different FID responses (Figs. 2, 5, 7) it should be possible to eliminate some of the variability in FID

responses by considering each rod as an isolated analytical unit. FID data were compiled in this manner for a comparison between the precision of FID responses after single and double developments (Table 3). Intrarod CVs were obtained from the response data for each Chromarod in the set. By taking the average of the 10 CVs, a mean intrarod CV representative of the rods under each set of developing conditions was obtained (Table 3).

CVs for classes analyzed on the first scan and for PL were improved with double developments in the same solvent system (Table 3). For the other classes, especially TG, the precision was worse after a double development. This may be related to the use of different development times in different solvent systems [Fig. 7(b)]. The second hexane-based solvent system used (98% hexane by volume) was considerably less polar than the first one (80% hexane). It is possible that the redevelopment performed in this system was not quite long enough and that the solvent system was not quite polar enough to ensure a uniform FID response for all scans of these compounds. This would be especially true for TG which is moved the furthest from the point of application in the first development [Fig. 7(a)].

The improvement in CV for PL (Table 3) is exceptional, considering that developed peaks have a lower area [Fig. 7(b)]. The improvement in standard deviation probably is related to the lack of mobility of PL in neutral lipid solvent systems [Fig. 7(a)].

TABLE 3

Mean Intrarod CV of FID Responses from 3 μg of each Lipid Class with 7 Analyses per Compound per Rod, Developed Either Once or Twice

Class	Single development[a]	Double development[a]
HC	13	12
WE	17	16
KET	9	9
FFA	20	16
TG	11	19
ALC	8	13
ST	12	13
PL	11[b]	9

[a]Typical chromatograms are shown in Figs. 6 and 7.
[b]PL remains at the point of application: Fig. 7(a).

TABLE 4

Mean CV of Interrod and Intrarod FID Responses and Ratios (italicized) from Double Developments

Class	Interrod precision		Intrarod precision	
HC	17	*14*	12	*12*
WE	14	*13*	16	*18*
KET	13	–	9	–
FFA	20	*14*	16	*17*
TG	16	*15*	19	*19*
ALC	15	*15*	13	*13*
ST	16	*12*	13	*14*
PL	14	*16*	9	*16*

Inter- and Intrarod Precision

If different rods elicit different responses as a result of the overall characteristics of individual Chromarods, then the use of an internal standard should reduce interrod variability. Similarly, the use of data from individual rods should also reduce the variability in analyses so long as rod conditioning is the same for every development. Table 4 indicates that differences in the characteristics of Chromarods are not the major cause of variability in the Iatroscan-Chromarod system.

Table 4 gives an intercomparison between interrod precision and intrarod precision for the same set of 10 Chromarods. The intrarod precision data is the same as that used for double developments in Table 3. Thus, there are seven analyses per compound per rod. The interrod precision in Table 4 is calculated from the same data set.

The FID responses of some compounds display a deterioration in precision when their areas are normalized to that of the internal standard. For some others the precision is worse if intrarod data is used rather than interrod data. This implies that the magnitude of FID responses for some compounds is independent of that of the internal standard, while for others the differences in FID responses among the 10 rods of a set during any given run is smaller than the variability from run to run. For the latter compounds there is some suggestion of a bimodal distribution of FID responses and of a correlation among the responses of these compounds. Thus, for any particular run, if the peak for WE on the first rod was notably higher than the mean for that compound on the previous run, then not only would the remaining WE peaks on the nine rods be high, but so would the TG and FFA peaks. This distribution was not discernible in the FID responses of the other compounds.

Although no detailed pattern emerges from Table 4, it is possible to make some tentative recommendations on the basis of these data. Since, on average, intrarod precision is at least as good as interrod precision, it should be possible to construct individual calibration curves

over relatively long time periods. This is useful when a large number of samples need to be analyzed, a common requirement in oceanography.

When KET is used as a 'surrogate spike' (21) for estimating the recovery efficiency of lipid classes from seawater samples (3), it cannot be used simultaneously as a reference to compensate for the variability in Iatroscan FID responses. This is because the different response ratios obtained from different loads [Figs. 2 and 3, (7,15)] would necessitate the recovery from seawater samples of identical amounts of KET for any given set of standards. Thus, for the determination of lipids in spiked seawater samples it is better to leave calibration data in integrator area units (3) than to normalize it to a fixed amount of KET (Figs. 2 and 3). Since intrarod precision is not improved by taking data in ratio to the response for KET (Table 4), this is not a drawback.

If only a few samples are to be analyzed, calibration curves can be constructed on the basis of interrod data, especially if a controlled amount of internal standard is added to each Chromarod. This is the most common method used for TLC/FID calibration (6,7,12,15,17). The use of an internal standard improves reproducibility for classes analyzed on the same scan [Table 4, (2,7)]. However, it is advantageous to use the unique partial scanning facility on the Iatroscan when complex lipid mixtures need to be characterized. Since normalization to KET produces the best improvements for interrod data obtained on the same scan (Table 4), an internal standard ideally should be included with every scan.

The most accurate assessment of marine lipid class concentrations should be obtained from a calibration scheme that permits an examination of both run to run and rod to rod differences. In effect this is a means of parameterizing the variability in the FID response originating at the detector stage, and the variability of the FID response that is a result of differences among Chromarods. Unfortunately, even with the most detailed calibration schemes, it is unlikely that the Iatroscan-Chromarod system, in its present design, will ever approach the precision currently enjoyed by other chromatographic techniques. However, recent innovations in the Iatroscan system (22,23) should begin to bridge this gap.

In its present format, the Iatroscan-Chromarod system is likely to be more than adequate for many oceanographic studies. Problems associated withshipboard laboratories, sampling and operationally defined 'fractions' of organic matter (24) are a more serious impediment to the collection of precise data than are most analytical techniques. Detailed analyses of minor organic compounds in seawater often are of secondary importance to oceanographers. Of greater interest is an assessment of the more fundamental interactions between classes of marine compounds and biological and physical processes occurring in the water column.

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