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Review

Thin-layer chromatography–flame ionization detection Iatroscan system

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ABSTRACT

The thin-layer chromatography–flame ionization detection (TLC–FID) Iatroscan system is a technique which is still being evolved. Quantification with the TLC–FID system relies heavily on the accurate setting up and calibration of the instrument. An appreciation of the factors that influence the analysis can eliminate significant errors. At least a few of the numerous operating variables need to be fixed to obtain coherent results from different laboratories. Hydrogenation of the sample is recommended in order to improve quantification with the Iatroscan system. The improved reproducibility obtained with automatic sample spotters compared with manual spotting indicates that autosampling is highly advisable.

CONTENTS

1. INTRODUCTION

The Iatroscan is an instrument that combines the resolution efficacy of thin-layer chromatography (TLC) with the possibility of quantification by flame ionization detection (FID). This instrument is becoming increasingly popular in the field of food science, where it is being used extensively for lipid analysis. When the instrument was first marketed, more than two decades ago, there were controversies regarding the acceptability of the results obtained [1,2]. The major drawback of the instrument was a lack of quantitative accuracy and reproducibility. Relative standard deviations (R.S.D.s) as high as 30% and 83% [3,4] have been observed, especially for low sample loads. Since then, a number of papers have been devoted solely to the means of understanding the full working of the instrument and to increase its applicability in quantitative analysis [5–9]. This resulted in a better understanding of the working of the instrument, improved instrument design, especially the detector (making it more sensitive and reproducible), newer and more uniform Chromarods (the TLC component), better techniques for sample application, etc. Several reviews [5,7–9], an exclusive book [6] and a symposium volume on Iatroscan [10] have further helped in building the image of this instrument.

However, in spite of these several reviews and a better understanding of the instrument, it is not unusual still to find statements such as "the application of this method in lipid metabolism research tends to be limited" [11]. Whereas in a review in 1990, quantification of samples spotted on Chromarods without being subjected to solvent development (discussed later) was considered as irrelevant [7], in a more recent study such a technique was used for the determination of total lipids [12]. In two papers published in the same issue of a journal in 1991 there are contradictory statements regarding a particular aspect of the instrument (discussed later).

What is plaguing this instrument and why, even after two decades following its introduction, has it not found greater favour with analytical researchers and not yet been more widely adopted in quality control laboratories? With this background, it is not surprising that the TLC–FID system receives scant mention in *Chemical Abstracts* and *Analytical Abstracts*. This is because the users of this instrument generally tend to fall into two categories: those who routinely use it for quantification purposes and find it satisfactory, and others who have certain doubts regarding the acceptability of the results obtained. In an effort to make this instrument appear acceptable, most papers and reviews tend to overlook its drawbacks.

One of the disadvantages with this instrument is that it has several operating and user variables, only a few of which have been fixed by the manufacturers. A change in even one of the variables affects the results, which, in part, accounts for the conflicting observations from different laboratories. This necessitates a thorough standardization of the instrument and the procedural work, and, most of all, an understanding of the working of the instrument.

In this review some emphasis will be placed on understanding the instrument. The various aspects of which one needs to be aware when using the instrument will be treated in an uncomplicated and simple manner. The literature comparing TLC–FID with standard instrumental techniques will be covered and some applications of TLC–FID to foods, with special reference to the oil and fat industry, will be discussed.

2. INSTRUMENTATION

The Chromarod Iatroscan system consists of two independent units, the Chromarods which constitute the TLC component and the Iatroscan, the FID scanner unit. An early review by Ackman [5] and an excellent book on the TLC–FID Iatroscan system

[6] give a thorough illustration of the instrument. Therefore, only a brief discussion of the instrument will be given here.

The Chromarod is a quartz rod with a diameter of 0.9 mm and a length of 152 mm. A 148-mm length of the rod is coated with a thin layer (75 μm) of a mixture of soft glass powder and the adsorbent, either silica gel (Chromarods S, SII and SIII) or alumina (Chromarod A). These differ in the nature of the absorbent or its particle size [7]. The recently introduced Chromarod SIII is the only format now available [13].

The Iatroscan FID scanner consists basically of a hydrogen flame jet and an ion collector. The sample is burnt, the ions are collected by the collector electrode and the signal is amplified in a similar way as in the gas chromatography (GC)–FID.

The newer models of the instrument, the Mark IV and Mark 5, have an improved detector [7,8]. The ion collector is in close proximity to the Chromarods, making it a more efficient ion collector. The modified detector in the newer model was illustrated schematically in an recent review [7]. The Mark 5 is an improvement on the Mark IV in terms of operational features, doubled amplification to improve the detection limits, a built-in air pump unit and a decreased instrument mass [14].

The validity or accuracy of the TLC–FID method is a question of using the most suitable response factor (correction factor) for the sample. Hence it will be useful to consider the various factors that could affect the response of the flame ionization detector of the Iatroscan system.

3. FACTORS THAT AFFECT THE FID RESPONSE OF THE SAMPLE IN THE IATROSCAN SYSTEM

3.1. Chromarods

Chromarods are the heart of the TLC–FID Iatroscan system. The Chromarods come in a set of ten that can be mounted on a frame. The FID responses are shown to vary between the different sets of Chromarods and also from rod to rod within a set. Its has been suggested that one should match and select ten Chromarods having similar characteristics from a larger batch, in order to avoid rod to rod variation [7]. The grouped set of ten rods should then be treated in a similar fashion. Al-

though this practice is desirable, it is expensive [a set of ten Chromarods SIII cost ca. US \$215 (RSS, CA, USA)] to buy large numbers of Chromarods and moreover the procedure is time consuming. In such instances it is more advisable to consider each rod as a single isolated analytical unit and construct a calibration graph for each rod [7]. This may appear to be tedious but is a definite advantage as results can then be expressed with more confidence without any problem of rod to rod variation. The newly introduced SIII rods, being machine made, are more uniform. Sebedio and Juaneda [15] contend that the rod to rod variation among the SIII rods is low and hence it is not necessary to treat each SIII rod as a entity. However, it is essential to test a newly opened set of rods before assuming any such uniformity.

3.2. Nature of the sample

The response of a substance in TLC–FID is strongly affected by its composition. Hydrocarbons have the highest ionization abilities, whereas compounds containing heteroatoms such as oxygen, phosphorus or halogens give a lower response. Sterols are reported to give a high response, probably owing to the planarity of the molecule [7]. Even within the same lipid class the response varies depending on the molecular complexity of the substance. Excellent examples are natural samples such as fish oil triacylglycerols (TAGs), which give a much lower response than an equivalent mass of pure standard triolein. The multiplicity of the fish oil TAGs results in partial subfractionation on the efficient Chromarods. This results in shoulders or broadened peaks which give a lower response. Fraser and Taggart [16] observed a 1.7 times higher response for triolein compared with an equivalent amount of fish oil (Marinol) triacylglycerols.

The various suggestions in the literature concerning this discrepancy include the following: (a) if the peak is too broad, it will be advantageous to use a slightly more polar solvent; the subfractionation effects are not as marked in a polar solvent, hence a narrower peak could be obtained (Fig. 1) [13]; (b) double development in the same or a different solvent system can sometimes improve the FID response by producing a narrower band on the Chromarod [3]; (c) the use of reference standards more
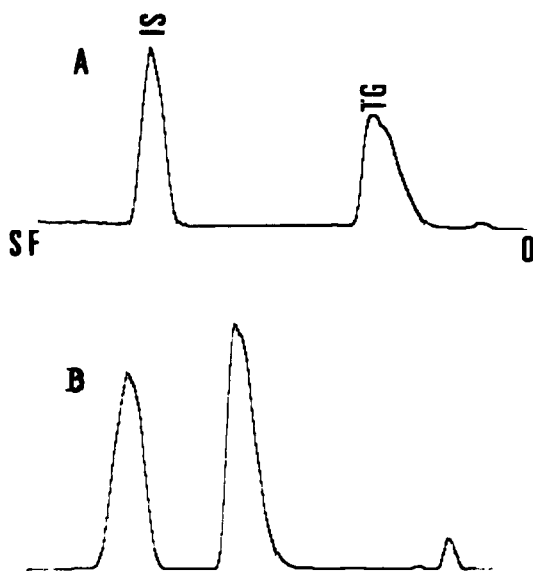
Fig. 1. TLC–FID showing the effect of the developing solvent on the peak shape of fish oil triacylglycerol on Chromarods SIII. (A) Developed in solvent system hexane–diethyl ether–formic acid (97:3:1, v/v/v); (B) developed in solvent system hexane–chloroform (85:15, v/v); the chloroform contains 5% of 2-propanol and 0.5% of formic acid. IS, internal standard, wax ester; TG, triacylglycerols; O, origin; and SF, solvent front. From Shantha and Ackman [13].

closely resembling the composition of the substance being analysed for TLC–FID calibration purposes [16]; purified natural standards or, when not available, synthetic standards could serve the purpose, but such standards can be expensive and unsaturated standards are less stable; (d) hydrogenation of the sample (discussed later) also produces an increase in the response by removing subfractionation effects due to the presence of an unsaturated system [13,17–19].

3.3. Amount of sample spotted and linearity of response

The response factor of any lipid class depends on the amount of sample spotted. The non-linearity of the FID response in the TLC–FID system even within a small working range comes as a shock to those accustomed to the FID response in GC. The suitable working range of the sample size is one in which the FID response is linearly dependent on the sample amount. Most calibration graphs of FID response versus sample mass obtained with the earlier instruments, equipped with the older detector, ex-

trapolated to negative intercepts on the ordinate, making it unsuitable for low concentrations (< 1 μg). In such cases of very low sample concentrations, as frequently encountered in aquatic samples, multi-level extensive calibration graphs at such low sample loads need to be constructed before the results can be considered acceptable [3].

The later Mark IV and Mark 5 models, with an improved detector design, are claimed to give good linearity over the working range up to 25 μg, with as little as 2.5 ng giving a measurable response [7]. However, in order to obtain a reasonable signal-to-noise ratio, it is recommended that sample amounts should exceed 10 ng [14]. Fig. 2 gives the calibration graphs for different lipid classes taken in the ranges 20–10 240 and 20–640 ng [15]. The Mark IV model, with an improved detector and the new Chromarods SIII, were used in this study. Note that the graphs are not linear over the range studied and that the response of the triglyceride (trilinolein) is different from that of cholesteryl ester.

Fig. 3 gives calibration graphs for three lipid classes [8]. The samples were developed on Chromarods SIII and the Mark IV model was used for scan-
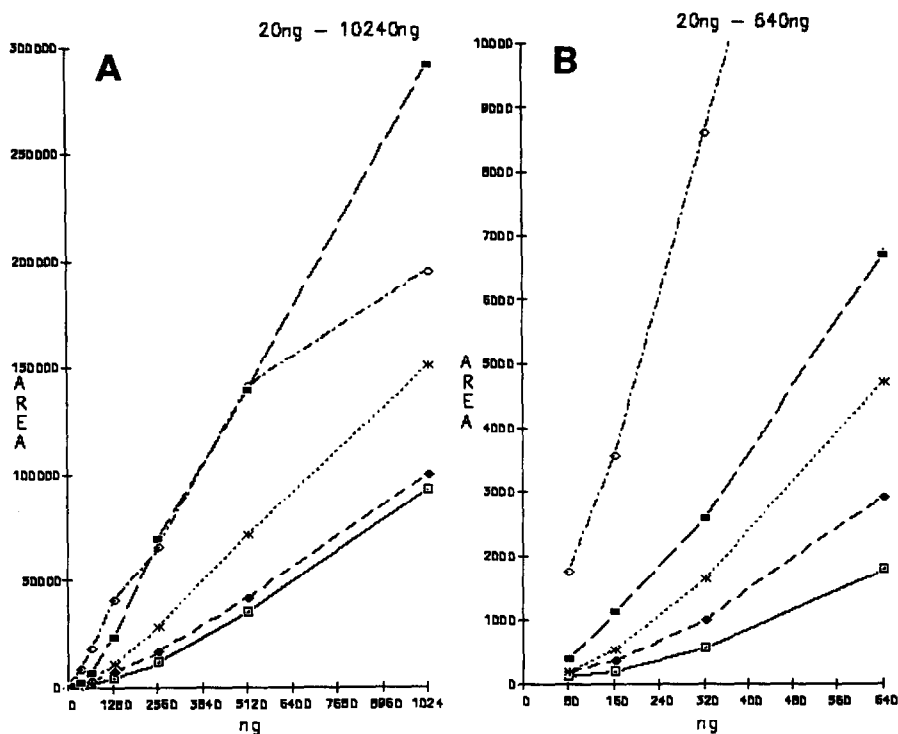
Fig. 2. Mass area response for (□) trilinolein, (◆) linoleic acid, (×) cholesterol, (○) cholesterol ester and (■) phospholipids developed on Chromarods SIII and scanned in the later model of the Mark IV Iatroscan. (A) Sample mass from 20 to 10 240 ng; (B) mass from 20 to 640 ng. From Sebedio and Juaneda [15].

ning. The graph is linear, the response of cholesteryl ester and triglyceride (species not shown) having similar responses. Of course, an exact comparison of Figs. 2 and 3 cannot be made, as little of the operating history is known. This is just an example of where two laboratories differ regarding the linearity of the response obtained from the newer improved detector of the Mark IV instrument and using the more uniform Chromarods SIII. Both studies [8,15] agree that the sensitivity and reproducibility have been improved with the later Mark IV model.

3.4. Sample preparation, spotting technique and conditioning of Chromarods

The sample needs to be dissolved in a suitable solvent before application on the Chromarods. Chloroform–methanol (5:1 or 2:1, v/v) is suitable for the dissolution and application of lipid samples on the Chromarods.

The spotting technique is a major factor that effects quantification with the Iatroscan. A large drop spotted on the Chromarod would spread on the rod, the spreading being more pronounced with alumina than silica rods [6]. This would result in a broadened band with a lower FID response. A sufficiently concentrated solution can be applied in a single aliquot of up to 0.5 μl. With dilute samples where relatively large volumes (10 μl) need to be spotted, it should be spotted in repeated aliquots allowing for drying of the solvent between each application. In such instances a drying lamp or a hotplate can be used to dry the solvent. In any event, the spreading of the solvent is inevitable when spotting such large volumes. Solvent focusing [3,20], i.e., developing the rods in a polar solvent, such as acetone, to just above the point of application would refocus the spread out solute into a narrow band. A Drummond Microcap disposable pipette (1–10 μl) can be used for spotting; the use of a syringe fitted with a repeating dispenser or an automatic applica-
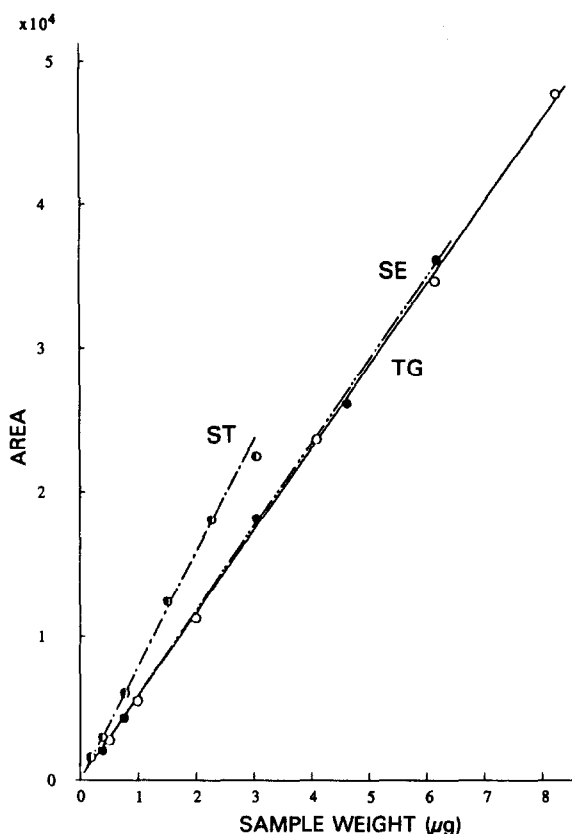
Fig. 3. Mass area response for three neutral lipid derivatives in hexane–diethyl ether (6:1, v/v) on Chromarods SIII and scanned in the later model of the Mark IV Iatroscan. TG, triacylglycerols; ST, cholesterol; SE, cholesteryl ester. From Iatron Laboratories, Tokyo, and Ohshima and Ackman [8].

tor is more suitable. Read [21] compared the different spotting techniques and suggested an improved rotary sample application method for the Iatroscan. In a recent paper, Sebedio and Juaneda [15] report a better R.S.D. (6.6%) for samples spotted using a autospotter than for manually spotted samples (10.1%).

The activity of the adsorbent on the Chromarods is strongly effected by the relative humidity of the laboratory atmosphere. The rods absorb moisture at a high rate, producing drastic changes in the chromatographic behavior of the rod. The few minutes during which the rod is exposed to the laboratory atmosphere during spotting could produce a decrease in the reproducibility of separation and response. It is recommended that the rods be dried

in a desiccator for 5 min to remove absorbed moisture prior to developing in the solvent [7]. Alternatively, they can be conditioned at constant humidity (e.g., 30%) by placing them in a closed chamber containing saturated salt solution [3,7]. The main aim of these treatments is to make the Chromarods have similar properties that can be easily reproduced during experiments, thereby allowing more consistent results.

3.5. Developing solvent and relative position of the sample on the Chromarod

The response of any lipid class is affected by the distribution of the sample along the Chromarod (Fig. 4) [3,7,22]. The sample material spotted on the Chromarod and subjected to development in a solvent is affected by the extent of its exposure to the solvent. A spot located nearer to the origin, which has solvent constantly passing through it, gives a
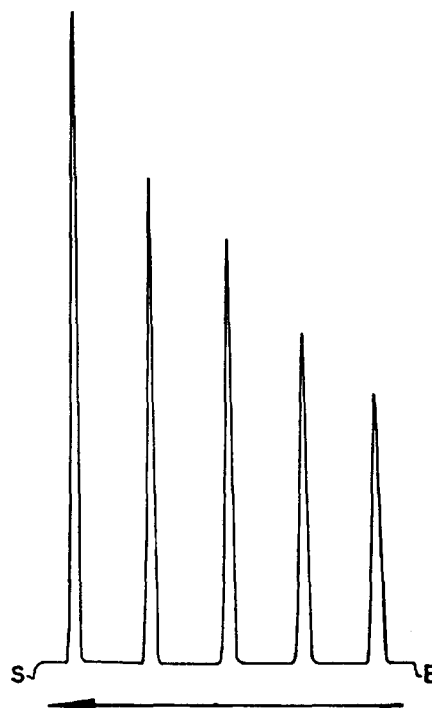

Fig. 4. TLC–FID showing the relationship between the distribution of the material (hexadecanone) on the Chromarod and the peak shape, and thus the response. The rod was developed for 40 min in hexane; S and E denote the start and end of the scan. From Parrish and Ackman [22].

short and broad peak having a low response. Variations in response could also arise from the non-uniformity of the adsorbent layer on the rods, the rods not being isotropic along their length. Kramer *et al.* [12] recently observed a significant positional effect in the FID response of methyl heptadecanoate spotted at nine different positions on the Chromarod, which was not subjected to any solvent development. This suggests that the positional variations are not necessarily due to solvent exposure, but could also be due to the anisotropy of the Chromarod. The newer Chromarods SIII, which are machine made with a more uniform adsorbent layer, need to be assessed for this effect.

In a few studies the sample material was analysed by FID without subjecting it to development in any solvent. Although this is known to result in split peaks, Kramer *et al.* [12] did not observe any such peak splitting of an undeveloped sample. They observed a large decrease (about 46%) in the FID response for the same amount of lipid sample on the same rods following development. This necessitates that the two techniques of analysis, *i.e.*, analysis of sample spotted on Chromarods that have been subjected to solvent development and analysis without subjecting them to any such development, be considered as separate studies. The solvent chamber has to be saturated with the solvent before inserting the rods for development. The eluent system and developing time (as the solvent front is not very visible) should be strictly adhered to in order to obtain reproducible results. The developing time depends on the solvent used and needs to be standardized in each laboratory. The temperature at which the rods are developed should also be maintained constant, as this has been shown to affect the response by producing a change in the R_F values [3]. A wealth of literature is available on the solvent systems to be used to effect the separation of a variety of samples [5–7] and details will not be repeated here.

3.6. The FID unit

3.6.1. Gases

The response of the detector varies with the flow-rate of hydrogen, although it is not as drastically affected by the flow-rate of air. Flow-rates of 2000 ml/min for air and 160 ml/min for hydrogen are generally recommended [5]. However, many studies show that a higher rate of 173 ml/min [3] or 180 ml/min of hydrogen gives better response and reproducibility [7]. Hence, depending on the nature of the sample, the flow-rate of the FID gases can be adjusted to obtain the optimum response and better reproducibility. Caution is needed in increasing the hydrogen flow-rate, as extreme heat may damage the frit of the Chromarod and shorten its life. High purity of the FID gases is a prerequisite for minimizing the baseline noises.

3.6.2. Scan speed

The response of the FID is dependent on the rate at which the rods pass through the flame, *i.e.*, the scan speed. In later models, the instrument is provided with a speed selector with numbers ranging from 1 to 5 and with scan speeds ranging from 2.5 to 5.1 mm/s. The recommended scan speed should allow complete combustion of all components while still being fast enough to avoid unnecessary thermal damage of the Chromarods [5,6].

3.6.3. Configuration of the detector

The response and reproducibility vary with the position of the Chromarods with respect to the flame and the ion collector. Przybyiski and Eskin [23] have modified the existing detector of the Mark II instrument to improve its sensitivity, linearity of response and stability. They adjusted the ion collector closer to the Chromarods (0.8 mm above the rods) and installed a ball electrode inside the ion collector. Kramer *et al.* [24] observed a fourfold increase in response when the ion collector was adjusted 1.7 mm above the rods rather than with the recommended space. In the newer models (the later Mark IV and Mark 5) the ion collector is in close proximity to the Chromarods, making it a more efficient ion collector. The detector is said to be more sensitive, with detection limits as low as 2.5 ng, and to give better reproducibility [8]. The linearity of response over a wide sample range, however, needs to be studied further. It will be useful if users of the newer model can provide information on the reproducibility and linearity of response that can be obtained with the instrument. The introduction of the commercially available flame thermionic ionization detector (FTID), which can be fitted on the older models, is useful for specific detection of nitrogen and halogen compounds [25,26].
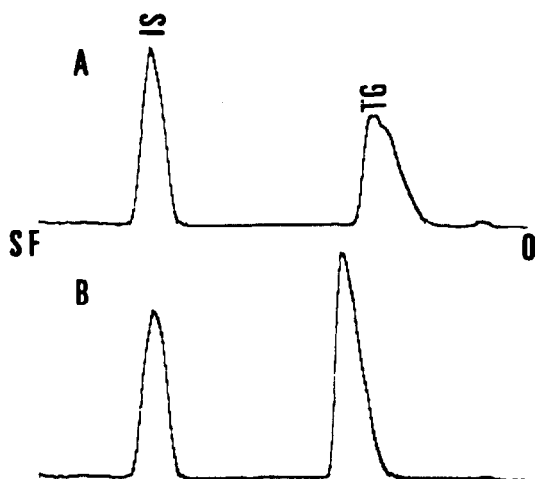
Fig. 5. TLC–FID showing the effect of hydrogenation on the peak shape of fish oil triacylglycerols (TG) on Chromarods SIII. (A) Unhydrogenated; (B) hydrogenated, developed in solvent system hexane–diethyl ether–formic acid (97:3:1, v/v/v). IS = internal standard; SF = solvent front. From Shantha and Ackman [13].

4. TECHNIQUES DEVELOPED TO IMPROVE ANALYSIS BY TLC–FID

4.1. Hydrogenation

Hydrogenation of unsaturated samples prior to TLC–FID has been shown to produce an increase in the FID response [13,17–19]. Hydrogenation can be performed in the laboratory by passing hydrogen through the sample in a suitable solvent and in the presence of platinum oxide (Adam's catalyst) with constant stirring. A detailed description of the hydrogenation procedure is given elsewhere [13]. The advantages of hydrogenation include (a) greater stability of the sample, thereby allowing for its analysis at a later time; (b) an important and useful increase in response for most lipid classes, including those containing only one double bond (the increase in response following hydrogenation was as high as 45% in the case of fish oil triacylglycerols [13]); (c) better peak shapes, as the subfractionation effects due to the presence of double bonds is nullified following hydrogenation (Fig. 5) [13]; (d) a better separation of lipid components in most instances, with a few exceptions (Fig. 6) [13]; (e) a lower R.S.D., i.e., better precision, than for unhydrogen-

ated samples; and (f) a simpler choice, lower cost and higher stability of standards that need to be used as references.

Exposure of Chromarods to iodine after the development of a sample was shown to increase the response [27]. Although this tends to saturate the double bonds by the addition of iodine, it is not as advantageous as hydrogenation and has not found many applications.

4.2. Impregnation of the Chromarods

4.2.1. Copper(II) sulfate impregnation

The response of a lipid class is higher on the impregnated rods than on unimpregnated rods [14,28–30]. Copper(II) sulfate could bring about a more uniform ionization of the sample, which could be lost by simple volatilization/pyrolysis using unimpregnated rods. Moreover, the copper could react with the organic compound at high temperatures of the flame, giving an organometallic compound, with an increased response [28]. The various advantages of using copper(II) sulfate-impregnated Chromarods for analysis include (a) an increase and a more uniform response for most lipid classes, (b) better reproducibility, (c) minimization of rod to rod variations, (d) better visibility of the solvent front and (e) improved baseline stability. The resolution of phospholipids is said to fall following impregnation of the Chromarods with copper(II) sulfate [30,31]. Hence a change in the composition of the developing solvent from that used when using unimpregnated rods is necessary to obtain optimum resolution.

4.2.2. Boric acid impregnation

Impregnation of Chromarods SII with 3% boric acid solution has been used for the complete separation of glyceride hydrolysis mixture (triolein, 1,3- and 1,2-diolein, 1-monoolein and oleic acid) [32]. Boric acid impregnation has been used to effect resolution between mono- and diglycerides and isomeric polyhydroxy fatty acids [33]. The principle of this separation is the same as that in silica gel TLC.

4.2.3. Silver nitrate impregnation

Impregnation of Chromarods with silver nitrate has been used to separate and determine geometric and positional isomers of fatty acids and different
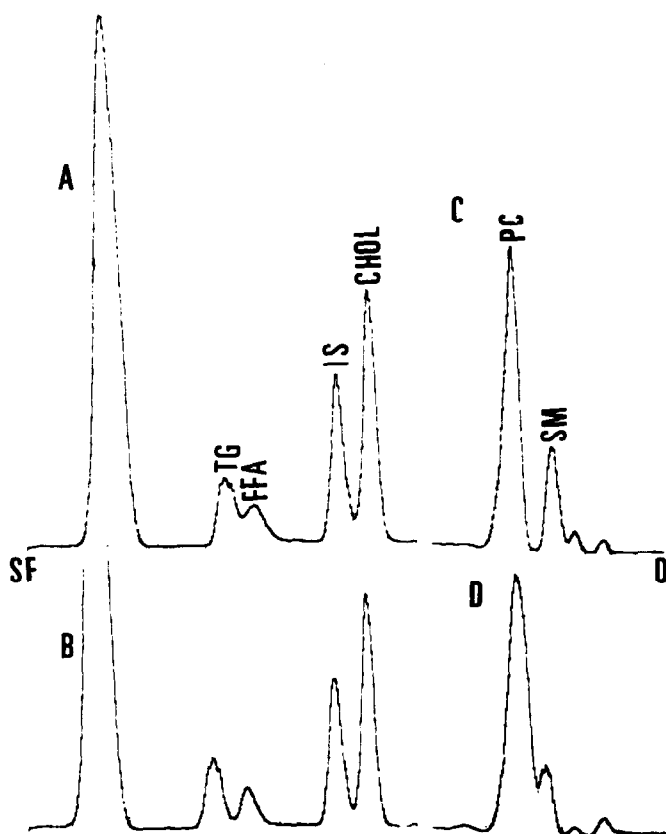
Fig. 6. TLC–FID showing the effect of hydrogenation on the peak shape and separation of human plasma lipids on Chromarods SIII in solvent system hexane–diethyl ether–formic acid (90:10:1, v/v/v), partial scan followed by complete redevelopment for analysis of polar lipids in solvent system chloroform–methanol–water (70:30:3.5, v/v/v). (A) Neutral lipids, unhydrogenated; (B) neutral lipids, hydrogenated; (C) polar lipids, unhydrogenated; (D) polar lipids, hydrogenated. SE = steryl esters; FFA = free fatty acids; IS = internal standard, fatty alcohol; SM = sphingomyelin; PC = phosphatidylcholine; TG = triacylglycerol; O = origin and SF = solvent front. From Shantha and Ackman [13].

species of triacylglycerols [34–36]. The rods need to be freshly impregnated before each analysis.

4.2.4. Oxalic acid impregnation

Oxalic acid impregnation of Chromarods is used to give an improved separation of phospholipids [37,38]. Impregnation with oxalic acid resulted in improved peak shapes and gave a good separation between phosphatidylinositol and phosphatidylserine. The oxalic acid concentration in acetone was found to be critical; concentrations lower or higher than 0.01 M were not satisfactory [38]. The Chromarods need to be impregnated each time before use.

A general note to add here is that with the newer type of instrument and more efficient Chromarods now available, impregnation of the rods should be done only if absolutely essential. When using these impregnation techniques the question arises of how often the impregnated Chromarods can be used before they need to be impregnated again. Silver nitrate-impregnated Chromarods can be used only once. Boric acid-impregnated rods have been used five times before renewing the impregnation [33]. Copper(II) sulfate-impregnated rods can be used for up to 25–30 scans [28]; more recently, Ranny et al. [31] observed that the rods need to be impregnated every few analyses, at least for the analysis of acidic phospholipids. The phospholipids seemed to react with the copper(II) sulfate. A white spot was

observed on a brown Chromarod at the site where phospholipid was present, after combustion [28]. Silver nitrate impregnation of the Chromarods is said to decrease their lifetime [6].

5. COMPARISON OF TLC-FID WITH CONVENTIONAL METHODS

From time to time a number of studies have been carried out using TLC–FID for various analyses and the results compared with those obtained with conventional methods. As no review has specifically covered this topic, it is hoped that this section will effectively provide information as to whether one can substitute an often time-consuming and elaborate conventional method with the rapid TLC–FID Iatroscan method.

5.1. TLC–FID Iatroscan vs. spectrophotometry for determination of phosphate value

Table 1 gives representative figures taken from two different studies [23,39]. In study 1 [39], the phospholipids (PLs) of oil were separated by column chromatography. The PLs were then applied

TABLE 1

COMPARISON OF TLC–FID AND SPECTROPHOTO-METRIC METHODS FOR THE DETERMINATION OF PHOSPHORUS CONTENT IN PROCESSED OILS

Plant oil sample	Phosphorus content (ppm)	
	Iatroscan	Spectrophotometry
Canola oil[a]		
Solvent	489 ± 5	506 ± 5
Expeller	172 ± 3	184 ± 3
Degummed	40 ± 2	42 ± 2
Corn oil[b]		
Crude	245	296
Degummed	4.9	9.3
Refined	0	2.5
Peanut oil[b]		
Crude	536	543
Degummed	3.1	35
Bleached	2.1	24
Refined	0	27

[a] Adapted from Przybyiski and Eskin [23].
[b] Adapted from DuPlessis and Pretorius [39].

to the Chromarods SII and developed in chloro-form–methanol–water-based solvent system and scanned in an Iatroscan Mark III. The phosphate content was calculated as $\mu g/g$ oil for individual PL peaks using a modified equation [39]. The phosphate content of each PL was then pooled to give a total phosphorus content. As a reference method the phosphate content was measured by means of the ash–spectrometric method [40]. In study 2 [23], the PLs were separated by two-dimensional TLC and analysed in a similar manner as in study 1 by TLC–FID. However, the detector of the Iatroscan Mark II was modified to give better sensitivity, stability and linearity of response [23]. Copper(II)-impregnated Chromarods were used. The ash–spectrophotometric method was used as the reference method. It is apparent that in both studies there is fair agreement between the phosphate value obtained using both the TLC–FID and the spectrophotometric methods, although the former gives slightly lower results. The higher values obtained by the spectrophotometric method has been suggested to be due to the presence of non-phospholipid phosphorus such as inorganic phosphates.

Other comparative studies include the PL compositions of trout gills [41] and rat heart [24]. Hazel [41] concluded that the results obtained by TLC–FID compared well (within 5%) with the traditional phosphate analysis for all PLs except phosphatidylinositol, where the values obtained from Iatroscan method were significantly higher (about 55%). No reason was given for this observation, however. Kramer et al. [24] studied rat heart lipids and concluded that the results obtained by the TLC–FID and the spectrophotometric methods for phosphate analysis were comparable, the standard error in the Iatroscan method, being higher, however, owing to wide variations in the results obtained.

5.2. TLC–FID Iatroscan vs. GC

5.2.1. Lipid analysis

Ranny et al. [42] compared the determination of commercial molecularly distilled acylglycerols by GC and TLC–FID methods. The results were found to be comparable with roughly the same reproducibility. The TLC–FID method was found to be preferable for serial analysis in process control or technical quality control. In a comparison of the

reproducibility of results obtained by the two techniques for the analysis of blood lipids, Mares et al. [43] concluded that the variations obtained in the Iatroscan analysis were much higher than those obtained by GC and that the former method needs to be improved to give better reproducibility. Rao et al. [44] evaluated the use of the TLC–FID system to study the lipid composition of alcohol-induced rat fatty liver. In most instances the values obtained by TLC–FID compared well with those given by GC, but in one instance the value obtained by TLC–FID was almost double that obtained by GC.

Sebedio and Ackman [34] analyzed a synthetic mixture of methyl stearate, oleate, linoleate and linolenate on silver nitrate-impregnated Chromarods and compared the results with those obtained from GC analysis using an open-tubular Silar 7CP column. Comparable results were obtained by the two methods for all methyl esters except methyl linolenate, the amount of linolenate measured by TLC–FID being considerably higher (18.7 ± 1.4%) than obtained by GC (11.8 ± 0.1%) [34]. Regarding precision, four GC analyses gave more reproducible results than ten Iatroscan analyses.

Beaumelle and Vial [45] transesterified crude lipid extract of human erythrocyte using boron trifluoride–methanol and determined the resulting FAMEs, dimethylacetal and total cholesterol by both GC and TLC–FID. The results correlated

well, with TLC–FID giving lower precision than GC. They did not account for cholesterol methyl ether and cholestadiene, which are formed when cholesterol is reacted with boron trifluoride–methanol.

Table 2 gives representative figures taken from two different studies [46,47], wherein the sterol content obtained from GC and TLC–FID analyses are compared. Walton et al. [46] determined cholesterol in seafoods by TLC–FID and Kovac et al.'s GC method [48]. The unsaponifiables were spotted on Chromarods SII and developed with hexane–chloroform–formic acid. The results obtained with the Iatroscan with the use of cholestane as internal standard, without an internal standard and by GC were comparable. More recently O'Keefe [47] determined the sterols in caviar on alumina rods and compared the results with those obtained using Chromarods SIII and GC. The results from GC compared better with the alumina rods than silica when used without any correction factors [47]. It should be mentioned that GC is capable of resolving the different sterols, which is not the case with TLC–FID, hence only the determination of total sterols is possible by TLC–FID.

Bascoul et al. [49] compared the levels of polar oxysterols as measured by TLC–FID and GLC. The values obtained by TLC–FID analysis (341 ± 65 ppm) were considerably higher than those ob-

TABLE 2

COMPARISON OF TLC–FID METHOD (USING CHROMARODS SIII AND A) WITH GC FOR THE DETERMINATION OF CHOLESTEROL IN FISH PRODUCTS

Sample	Cholesterol content (mg per 100 g)		
	TLC–FID		GC
Atlantic cod (raw)[a]	15.27 ± 2.40		17.95 ± 2.70
Atlantic halibut (raw, frozen)[a]	24.77 ± 2.30		26.24 ± 2.61
Atlantic lobster meat (cooked)[a]	83.59 ± 5.02		101.12 ± 8.98
	Chromarods SIII	Chromarods A	
Caviar[b]			
Beluga	422 ± 43	378 ± 13	342 ± 47
Oestra	579 ± 29	455 ± 20	404 ± 36
Lumpfish	516 ± 67	469 ± 10	303 ± 28

[a] Adapted from Walton et al. [46].
[b] Adapted from O'Keefe [47].

tained by GC (124 ± 50 ppm). The authors are of the opinion that results obtained by TLC–FID were more correct as they agreed more closely with the cholesterol loss during heating (376 ± 37 ppm). However, GC is a better method for evaluating specific cholesterol autoxidation products owing to its better resolution capabilities.

5.2.2. Dimer acid components

Zeman et al. [50] and Fritz et al. [51] determined dimer acid components by TLC–FID and GC method. Both concluded that the results are comparable and that TLC–FID is a superior technique for dimerization process control owing to its speed and simplicity with no exhaustive sample preparation. Rao et al. [52] found the results obtained by Iatroscan to be comparable to those obtained by GC, and high-performance gel permeation chromatography for the determination of monomer, dimer and trimer contents in methyl esters of dimer acids.

The Iatroscan method was shown to give better precision than TLC–densitometry for the analysis of cosmetics [53]. Mills et al. [54], in an analysis of lipoproteins, were of the opinion that the results obtained using the Iatroscan were less accurate than those obtained by conventional methods.

The less accurate and less reproducible results obtained with the Iatroscan as compared with other methods could be due to the number of user and operating variables of the instrument. It could also be due, at least in part, to the lack of familiarity with this instrument. It can be envisioned that using an unfamiliar method would naturally lead to considerable variability in results as compared with an accustomed house method. The choice of a method in practice should be based on practicality, with the use of the least expensive and fastest method with the required degree of reliability.

At this juncture it is appropriate to pinpoint the advantages of the TLC–FID system over the conventional methods. The chief advantage of TLC–FID is the short analysis time, with the possibility of determining all components in a single analysis. The partial scanning and redevelopment [5–7] that can be done using the Chromarod Iatroscan system have no parallel in any other analytical technique. A good example of such a separation is illustrated in Fig. 7 [3]. As many as eleven classes of lipids were identified in marine samples using a combination of multiple developement schemes with the partial scanning facility of the Iatroscan. The ten Chromarods in a frame facilitate the analysis of ten samples at the same time. The high sensitivity of the improved detector permits the study of very small amounts (micrograms to nanograms) of sample. There is no need for elaborate sample preparation for TLC–FID analysis.
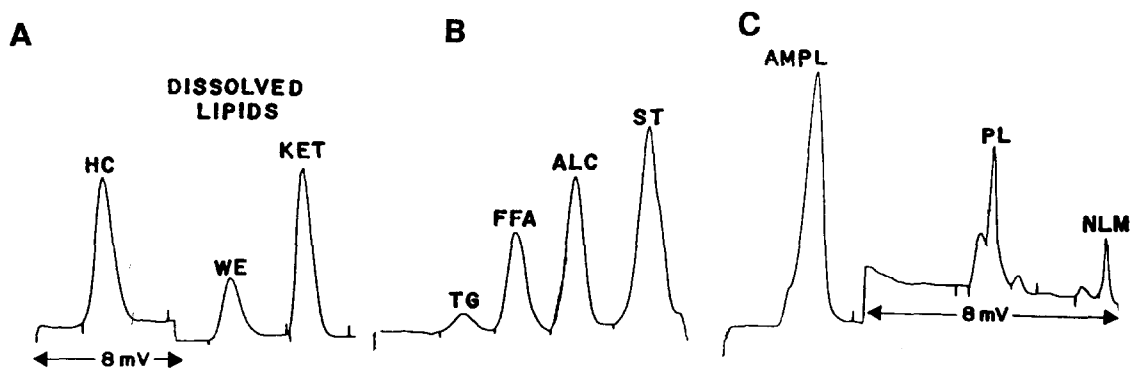

Fig. 7. Multi-class analysis of lipids using partial scan and redevelopment techniques. Each rod was scanned twice partially and once completely. (A) Double development in solvent system hexane–diethyl ether–formic acid (99:1:0.5, v/v/v), 25 min followed by 20 min, and scanned to the lowest point behind the ketone (KET) peak. (B) 40 min development in hexane–diethyl ether–formic acid (80:20:0.1, v/v/v) and scanned to the lowest point behind the sterol (ST) peak. (C) Last complete scan after double development first in acetone (15 min each development) and second in methylene chloride–methanol–water (5:4:1, v/v/v) developed for 10 min each. From Parrish [3]. Peaks: HC = aliphatic hydrocarbon, WE = wax ester, TG = triglyceride, FFA = free fatty acid, ALC = free aliphatic alcohol, AMPL = acetone-mobile polar lipids, PL = phospholipids, NLM = non-lipid material.

6. APPLICATIONS OF TLC–FID IATROSCAN SYSTEM

TLC–FID has widely been used in lipid analysis and this has been extensively reviewed [5–7] and will not be discussed further here. Only the few instances where TLC–FID has been applied to a specific problem in the oil and fat industry and which could be further developed to serve the food industry will be discussed.

6.1. Oils and fats analysis

TLC–FID finds wide application in the analysis of oils and fats [5–7]. Lipid contents in fish and deep-sea sediments have been determined using TLC–FID [20]. The sample, in chloroform–methanol (1:1, v/v), was spotted on the Chromarods. The entire sample was focused to one point by development in a polar solvent to just above the point of application. The sample was then scanned by the Iatroscan to give a total lipid measurement. A known standard having a composition similar to that of the sample was used as a reference to obtain accurate results [20]. More recently, Kramer et al. [12] determined the total lipids of Methanobacterium thermoautotrophicum using methyl ester as standard. The sample and standard were spotted alongside on the same copper(II) sulfate-impregnated Chromarod. The undeveloped rods were burnt as such in the flame ionization detector. The FID response factor of total lipids was assumed to be 1. The FID response of the standard was used to calculate the total lipid content [12].

TLC–FID has been used in industry for the analysis of castor oil [55]. The triacylglycerols (TGs) containing ricinoleic acid separate well from remaining TG species and can be easily determined. TLC–FID using silver nitrate-impregnated Chromarods has been used for the identification of various fats, such as beef tallow, olive oil and soya, cocoa butter, palm and coconut oils, rape, flax, corn and safflower oil [6]. TLC–FID with silver nitrate-impregnated Chromarods has also been used for determining trans fatty acids in margarine and partially hydrogenated oils and to study the methyl ester composition [34–36]. Kamata [56,57] determined the triglyceride composition of natural oils and fats such as lard, vegetable oils, fish oil and butterfat by the TLC–FID determination of their

oxidation products obtained following their partial hydrogenation.

The presence of high levels of cholesterol in foodstuffs is of major health concern. Walton et al. [46] determined the cholesterol level in fish products using TLC–FID and O'Keefe [47] that in marine-based food products (caviar) using alumina rods.

6.2. Oil refining

The presence of phosphatides in refined oil could cause stability problems. TLC–FID has been used to study the effects of refining on the distribution of phospholipids (PLs) in vegetable oil. The acetone-insoluble compounds from crude and degummed rapeseed oil were precipitated and the total PLs were determined by TLC–FID using 1,2-dichloroethane–chloroform–acetic acid (94.5:1:1, v/v/v) as the developing solvent [6]. The immobile PLs remaining near the origin were determined. DuPlessis and Pretorius [39] studied the total and individual phospholipids in refined canola oil using TLC–FID and using an equation to convert the PL content into phosphorus content. The Iatroscan method was found to be accurate in determining the PL phosphorus in the range 145–536 ppm; however, for lower levels (1–10 ppm) of PLs phosphorus the method was not accurate.

More recently, Przybyiski and Eskin [23] made a similar study of canola oil using copper(II) sulfate-impregnated Chromarods and modified Iatroscan Mark II detector. The improved sensitivity of the detector and the higher response generally obtainable on the copper(II) sulfate-impregnated rods allowed the determination of PLs and phosphorus in the nanogram range [23]. In the oil refining industry the TLC–FID Iatroscan system can be put to good use to study the extent of free fatty acids present and the saponification process.

6.3. Quality assessment of oils and fats

The presence of polar artifacts in fats, which is often due to oxidized fats, could serve as a suitable index to assess their quality. The oxidized material, being more polar, does not migrate on the Chromarods when hexane–diethyl ether–formic acid is used as the developing solvent and thus can be easily determined. Kaitaranta and Ke [58] studied the ox-

idation of fish oils, such as mackerel, herring and tuna. The increase in the signal of polar lipids was found to be proportional to the increase in the mass of the sample, although no such correlation was found when compared with the thiobarbituric acid value. Ranny [6] determined oxidized and unoxidized methyl linoleate by TLC–FID. They observed a direct relationship between the area of the oxidized peak and the peroxide value. Katoh *et al.* [59] determined the oxidation products in edible oil (corn, safflower) and methyl linoleate by subjecting the sample applied on Chromarods to three developing solvent systems, followed by scanning in the Iatroscan analyser. Beef tallow heated under deep frying conditions has been evaluated for cholesterol oxidation products using TLC–FID [49]. The extent of monomer, dimer and polymer contents in oxidized fat can also be assessed by TLC–FID [52]. These various studies suggest that TLC–FID can be used to study the oxidative deterioration of fats and oils.

6.4. Production processes and process control

TLC–FID analysis has been used in routine production processes and process control applications. It has been applied to study lipase kinetics, glycerolysis and esterification reactions [6,7]. Ranny *et al.* [42] found TLC–FID to be useful to study distilled monoacylglycerols. Ackman *et al.* [60] used TLC–FID to study the time required for complete esterification of fish oil triacylglycerols using methanolic sodium hydroxide as the transesterification reagent. Zeman *et al.* [50] and Fritz *et al.* [51] applied TLC–FID to dimerization process control. In view of its simplicity and rapidity, with no exhaustive sample preparation steps, TLC–FID can be exploited to advantage in routine production processes.

Other applications of TLC–FID in the food industry include analyses of sugars, carbohydrates, amino acids, food additives such as emulsifiers, flavors, preservatives and antioxidants and food contaminants such as plasticizers; these have been discussed elsewhere [6].

7. CONCLUSIONS

Quantitative analysis using the TLC–FID system relies heavily on the accurate setting up and cali-

bration of the instrument. An appreciation of the factors that influence the analysis can eliminate significant errors. One should remember that there are no experimental trivia in relation to Iatroscan analyses. It would greatly improve the image and acceptability of the TLC–FID Iatroscan system if the manufacturers in this field could come forward with models of Iatroscan instruments made in such a way that the entire detector can be replaced with a newer and more efficient detector. This would, at least in part, eliminate the cost of purchasing of a new instrument each time the detector is modified.

At least a few of the innumerable operating variables need to be fixed. For this, the positive points from various studies need to be taken into consideration, combined and assessed so as to give the best results. An inter-laboratory study using the best features could help in standardizing the technique for optimum results. From personal experience and from other studies, spotting is definitely one of the variables that needs to be standardized if reliable results are to be obtained. If the automatic sample spotter is as good as it is advertised to be, purchasers of the Iatroscan instrument should be encouraged to buy it. This may involve a higher initial cost, but would improve the accuracy and precision of the results by removing the large variations in results due to the spotting technique.

8. ACKNOWLEDGEMENTS

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