Flame photometric detector for thin-layer chromatography

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Abstract

A new flame photometric detector for thin-layer chromatography (TLC) was studied to determine sulfur and phosphorus containing compounds in materials with a high boiling point. The detector was integrated with a flame ionization detector into the Iatroscan TLC–flame ionization detection analyzer. The principle of the detector is based on the photometric detection of flame emission of heteroatom in a hydrogen–air flame. The emission spectra of sulfur and phosphorus were measured using dibenzothiophene (DT) and phosphoric acid as source materials. Interference filters of 394 and 526 nm were chosen for spectral isolation of the sulfur and phosphorus emissions. The effects of variation in air flow-rate and scan speed as related to both sulfur and phosphorus compounds were studied in order to define optimum detection conditions. The best result for the detection of DT as a sulfur compound was obtained under combined hydrogen and air flow-rates of 160 and 500 ml/min, respectively, with a scan speed of 30 s/rod. The response to DT was linear in the range of 0.25–4 µg. On the other hand, the most suitable conditions for detecting phosphatidylcholine (PC) as a phosphorus compound were combined hydrogen and air flow-rates of 160 and 1500 ml/min, respectively, with a scan speed of 40 s/rod. The response to PC was linear in the range of 0.25–16 µg. Application of the instrument with selective detection of sulfur and phosphorus compounds was demonstrated using heavy oils and human serum lipids.
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1. Introduction

Thin-layer chromatography (TLC) with flame ionization detection (TLC–FID) has been commonly used for hydrocarbon-type analysis. The application of TLC–FID has been reported for the analysis of lipids [1–5], petroleums (i.e. oil reservoir core extracts, condensates and oils plus heavy oils) [6–13], polymers [14,15], surfactants [16,17] and a variety of other materials with a high boiling point. Ackman et al. [18] in a detailed review provided an overview of analyses by Chromarod-Iatroscan TLC–FID. On the other hand, element-selective detectors are also necessary for characterization of compounds in complex mixtures. Flame thermionic ionization detection (FTID) was reported by Patterson [19] as an element-selective detection method for TLC. Nitrogen and halogen containing compounds, such as nitroaromatic compounds and chloro- and nitro-phenols, were detected by TLC–FTID. Indrasena et al. [20] also reported nitrogen detection of paralytic shellfish poisoning toxins by TLC–FTID. Holmes

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[21] developed a chemiluminescent detection (CLD) method for organic nitrogen detection and described one application of TLC–FID/CLD for determining the composition of oil shale bitumens.

There is currently no detector available for the selective detection of sulfur and/or phosphorus by TLC analysis. The selective detection of sulfur and phosphorus compounds in materials with a high boiling point, such as heavy oils and/or phospholipids would be helpful and possible using Iatroscan TLC methods.

Flame photometric detection (FPD) is well known as a detection method for gas chromatography (GC) [22–28]. The use of FPD for the selective detection of sulfur and phosphorus compounds was first introduced in 1966 by Brody and Chaney [22]. Since then, this detector has been used extensively for determination of sulfur and phosphorus compounds in the field of GC. The combination of GC and FPD is a very useful detection method. Addison and Ackman [29] established a rapid and sensitive method for measuring phosphorus in water, mud and biological samples by GC with FPD, and this method became an American Society for Testing and Materials method for measuring free elemental phosphorus. The FPD system is simple and has good output signal stability. Its main limitations are, however, related to a narrow linear range as compared to the typical 10^7 linear range of FID systems, and the fact that it is concentration sensitive, whilst FID is mass sensitive. This study describes the development of FPD with TLC–FID. Liner response curves of both sulfur and phosphorus compounds are obtained. The application of TLC–FID/FPD for monitoring sulfur and phosphorus compounds in materials with a high boiling point is described.

2. Experimental

2.1. Measurement of flame emission spectra

The Iatroscan newMK-5 (Iatron Laboratories, Tokyo, Japan) and the F-3010 fluorescence spectrophotometer (Hitachi, Tokyo, Japan) were used to measure flame emission spectra. By turning off the excitation lamp, the fluorescence spectrophotometer was used as a scanning monochrometer. A generated flame emission light in the Iatroscan was transmitted to the scanning monochrometer through a light guide made from a bundle of quartz glass fibers each 400 mm long and 4 mm in diameter. The Iatroscan operational conditions included combined hydrogen and air flow-rates of 160 and 2000 ml/min, respectively, with a scan speed of 40 s/rod. The scanning monochrometer was operated under the following conditions: emission band pass, 1.5 nm; scan speed, 120 nm/min; and response, 0.5 s. Dibenzothiophene (DT) and phosphoric acid (PA) were used as standards for sulfur and phosphorus. DT was obtained from Wako (Osaka, Japan), while PA was obtained from Kanto Kagaku (Tokyo, Japan). The samples were dissolved in hexane and water, respectively, before use. Chromarod-SIII rods (Iatron Laboratories) were used as stationary phase and for injecting sulfur or phosphorus compounds into the hydrogen flame. The entire Chromarod was immersed into the sample solution for 5 s. DT immersed Chromarods were dried at room temperature for 3 min, while PA immersed Chromarods were dried at 120 °C for 3 min in the TK-8 Rod Dryer (Iatron Laboratories). The Chromarods containing the samples were burned by the hydrogen flame of the Iatroscan, and the emission light from the hydrogen flame was measured through the light guide by the scanning monochrometer.

2.2. Description of TLC–FID/FPD system

A Iatroscan newMK-5 was modified for the trial manufacture of the new TLC–FID/FPD system. A schematic diagram of the TLC–FID/FPD system is shown in Fig. 1. A potential of 800 V was applied to a H6780 photomultiplier tube (Hamamatsu Photonics, Shizuoka, Japan) (1) which was used to detect the flame emission light. An electrometer (2) was used to transform the photoelectric signal current to voltage using a FET (field-effect transistor) input type operational amplifier. Narrow band pass interference filters (purchased from Opt-line, Tokyo, Japan) were used as optical filters (3). For sulfur compounds, a filter with maximum transmittance (30%) at 394 nm and a half-band width of 5.1 nm was selected, while for phosphorus compounds, a filter with maximum transmittance (51%) at 526 nm and a half-band width of 4.6 nm was selected. A
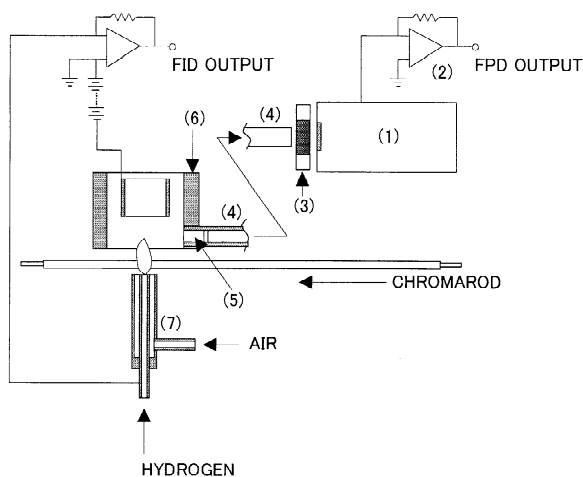
Fig. 1. Schematic diagram of the TLC–FID/FPD system.

light guide (4) with 40% transmittance at 394 nm was made of a bundle of 100 plastic fibers, each 400 mm long and 4 mm in diameter. The end of the light guide closest to the hydrogen flame was fixed onto a rod lens (5) for light collection and to protect the light guide from the heat of the flame. The end of the light guide was connected to a third electrode (6) above the hydrogen burner (7) in the Iatroscan.

2.3. Detection by TLC–FID/FPD system

The instrumentation described above was used to detect sulfur and phosphorus compounds and peak response was determined by integrating the peak area. The effects of variation in air flow-rates and scan speeds were studied using DT and phosphatidylcoline (PC) which contain one sulfur and one phosphorus atom per molecule, respectively. These commercial standards of DT and PC were obtained from Wako. First 4 μg of DT and 2 μg of PC were each spotted on to sintered silica-gel rods (Chromarod-SIII) with a Drummond micro dispenser. DT spotted Chromarods were developed in hexane (100%) at 20 °C for 15 min and then dried at room temperature for 3 min. PC spotted Chromarods were developed in choroform–metanol–water (40:20:1) at 20 °C for 15 min and then dried at 120 °C for 3 min. The Chromarods were scanned initially with a constant hydrogen flow-rate of 160 ml/min, an air flow-rate varying from 250 to 2500

ml/min, and with a constant scan speed of 30 s/rod for DT, and 40 s/rod for PC. The effects of varying air flow-rate on FPD responses were studied keeping hydrogen flow-rate and scan speed constant. The effects of varying scan speed on FPD responses were then studied by keeping the air flow-rate of 500 ml/min for DT, and 1500 ml/min for PC, and with a constant hydrogen flow-rate of 160 ml/min. A 394-nm interference filter was used for DT and a 526-nm filter for PC studies.

2.4. Response curves

DT and PC solutions of different concentrations (0.25–16 mg/ml) were prepared. A 1-μl aliquot of these solutions was individually spotted on to pre-scanned Chromarods. DT spotted Chromarods were developed in hexane (100%) at 20 °C for 15 min and after drying at room temperature for 3 min, the rods were scanned using the 394-nm interference filter. The operational conditions were 30 s/rod scan speed, 160 ml/min hydrogen flow-rate and 500 ml/min air flow-rate. PC spotted Chromarods were developed in choroform–methanol–water (40:20:1) at 20 °C for 15 min, then dried at 120 °C for 3 min and scanned with the 526-nm interference filter, with a scan speed of 40 s/rod, and with flow-rates of 160 ml/min of hydrogen and 1500 ml/min of air.

2.5. Applications

2.5.1. Heavy oil

A petroleum residue was used as a heavy oil sample, as nitrogen, sulfur and oxygen (NSO) containing compounds occur in higher concentrations in heavy versus light oils. NSO compounds occur mainly in the resin and asphaltene fraction in oils and are known to generate an FID response which deviates significantly from that of saturated and aromatic hydrocarbons [6]. The sample to be analyzed was dissolved in a dichloromethane to a concentration of 10 mg/ml, and 1 μl of the resulting solution was spotted on to Chromarods with a Drummond micro dispenser. The sample spotted Chromarods were developed in hexane (100%) at 20 °C for 15 min and then dried at room temperature for 2 min. The Chromarods were then developed in toluene (100%) at 20 °C for 7 min during the second

stage of development, and dried at room temperature for 2 min. In the third stage of development, the Chromarods were developed in dichloromethane–methanol (95:5) at 20 °C for 3 min. The rods were then dried at room temperature for 2 min and scanned using the 394-nm interference filter, at 30 s/rod scan speed, 160 ml/min hydrogen flow-rate and 500 ml/min air flow-rate. This procedure first allows maltenes to be isolated as saturated and aromatic hydrocarbons, and then the NSO fraction is split into resins and asphaltenes [6]. Note that asphaltenes remain on the point of application, i.e. asphaltenes are not eluted and are hence susceptible to the problem of rod rotation which influences results [6].

Rod rotation is, besides the correct response factors for individual petroleum fractions, the single most important factor which can ruin quantification of compound classes which are not eluted sufficiently up along the rod for distribution through diffusion, e.g. resins evenly around the circumference of the rod. Asphaltenes which do not move but remain at the point of application must always be oriented in the same direction with respect to the detector for valid quantification [6].

2.5.2. Human serum lipids

Human serum lipids were extracted from human serum by a modification of the Folch method [30]. A 0.5 ml sample of human serum was collected into a test tube and 10 ml of chloroform–methanol (2:1) was added. The test tube was shaken for 30 s by a vibration mixer and the solution was filtered. Then 2 ml of water was added to the test tube with the filtered solution and the test tube was shaken for 30 s. The solution was then centrifuged for 10 min at 830 *g*. The supernatant was removed and the residual solvents were evaporated by flowing nitrogen gas. The precipitated substance in the test tube was dissolved with 0.25 ml of chloroform–methanol (2:1) and 1 μl of the extracted solution was spotted on to the Chromarods with a Drummond micro dispenser. The sample spotted Chromarods were developed in chloroform–methanol–water–25% ammonia (47:20:2.5:0.28) at 20 °C for 43 min. Then, after drying at 110 °C for 3 min, the Chromarods were developed in hexane–diethyl ether (60:10) at 20 °C for 15 min in the second stage of development

and dried at 110 °C for 3 min. Finally the rods were scanned using a 526-nm interference filter, a scan speed of 40 s/rod, 160 ml/min hydrogen flow-rate and 1500 ml/min air flow-rate.

3. Results and discussion

3.1. Flame emission spectra

The emission spectra of DT and PA in the hydrogen flame are shown in Fig. 2. The emission from DT appeared as several peaks between 370 and 430 nm; the peak at 394 nm had the strongest emission intensity. On the other hand, the emission from PA had a strong peak around 526 nm. The molecular emission spectra due to the formation of S_2 and HPO species from sulfur and phosphorus compounds have been described in earlier work [22–26]. Our results are consistent with previous studies. The 394- and 526-nm interference filters were, as mentioned above, chosen based on the results for the TLC–FID/FPD system.

3.2. TLC–FID/FPD system conditions

Too great a hydrogen flow-rate in the TLC–FID system affects the linearity of the FID response curve [6,31]. However, the substances on the Chromarod cannot be completely burnt under lower hydrogen
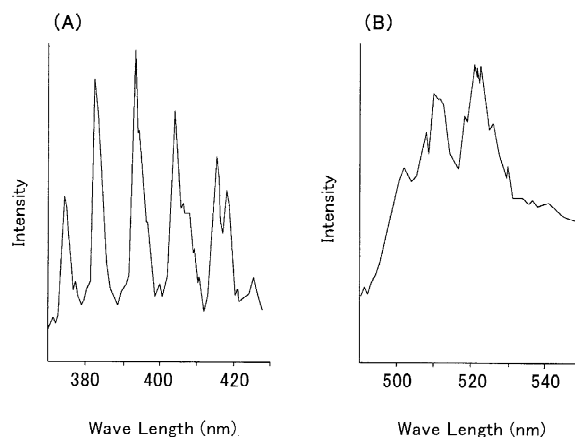

Fig. 2. Characteristic flame emission spectra. (A) Spectrum from dibenzothiophene; (B) spectrum from phosphoric acid.

flow-rates. Karlsen and Larter [6] found while using a large set of oils of varying American Petroleum Institute gravity and NSO content, that relatively high hydrogen flow-rates and medium scan speed were required to quantitatively liberate resins and asphaltenes from Chromarods. Hence it is clear that quantitative liberation of NSO compounds will affect FPD output. A constant hydrogen flow-rate of 160 ml/min was established in this study. The effects of varying air flow-rate and scan speed on FPD responses were studied, and are shown in Fig. 3. The scan speeds selected were 30 s/rod for DT detection, and 40 s/rod for PC detection. The intensity of DT

emission due to the formation of the S_2 species decreased with increased air flow-rate (A), so an air flow-rate of 500 ml/min was selected for optimum detection of sulfur compounds. Optimum intensity of PC emission due to HPO species was observed at an air flow-rate of 1500 ml/min (B).

The effects of scan speed are shown in Fig. 3C and D. Maximum DT emission intensity was obtained with a scan speed of 30 s/rod (C), and the intensity decreased with reducing scan speed. This suggests that DT began to vaporize from the Chromarod before light emission due to slow scan speed. Since DT is a volatile compound and as the
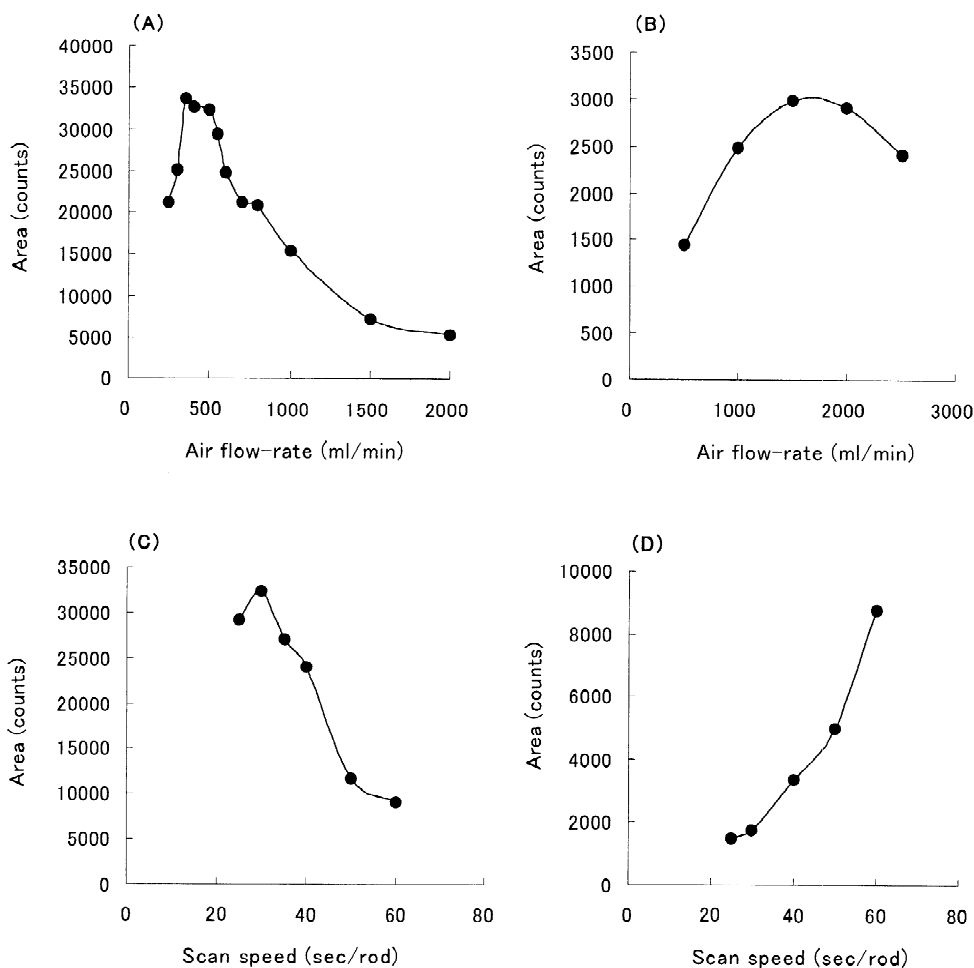

Fig. 3. Effects of varying air flow-rate and scan speed on detector response. (A) Effect of air flow-rate on the response of dibenzothiophene. (B) Effect of air flow-rate on the response of phosphatidylcholine. (C) Effect of scan speed on the response of dibenzothiophene. (D) Effect of scan speed on the response of phosphatidylcholine.
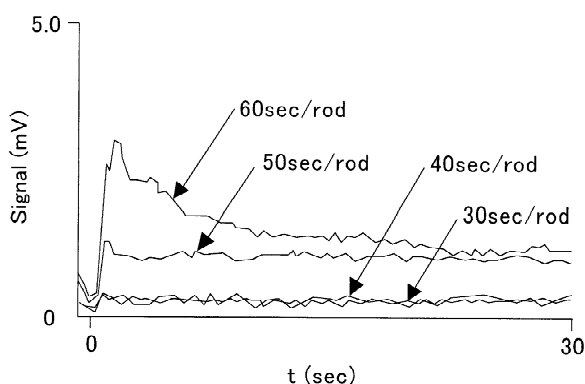
Fig. 4. Effects of scan speeds on the base line signals on the chromatograms.

temperature on the rod close to, but outside the reactive zone can typically attain 200 °C, this temperature will inevitably result in significant evaporation of compounds with high vapor pressures, e.g. light hydrocarbon compounds, whilst resins and asphaltenes are much less affected due to their very high boiling point [6]. On the other hand, the intensity of PC emission increased with reducing scan speed (D). PC as polar lipids interact strongly with the silica gel of the Chromarods, and it seems that more thermal energy is required for evaporation from the Chromarod. However, rising base line signals were observed on the chromatograms with

scan speed slower than 50 s/rod, as shown in Fig. 4. This suggests that silica gel and/or a glass binder as used in the Chromarod were vaporized by overheating and emitted weak light. In fact even a clean Chromarod glows intensely at scan speeds of 50 s/rod or slower. This intensive emission might influence the FPD system. It is possible that the lower scan speed causes a secondary unknown reaction due to overheating. Similarly, but using only FID, Karlsen and Larter [6] observed that the response of 12 selected standards and separated fractions from oils, i.e. saturated hydrocarbons, aromatic hydrocarbons and resins plus asphaltenes, systematically declined with slower scan speeds to a minimum at 40–50 s/rod before increasing again. Therefore, 40 s per scan was established for phosphorus compounds in the following studies.

3.3. Response curves

The responses to the sulfur and the phosphorus compounds of the FPD are shown in Fig. 5. The DT response curve due to S_2 emission is shown in (A), while (B) is the PC response curve due to HPO emission. Both responses to DT and PC are approximately proportional to the amounts of compounds spotted. The response of the FPD for GC is not linearly proportional to the amount of sulfur compound. In previous studies, Sugiyama et al. [26]
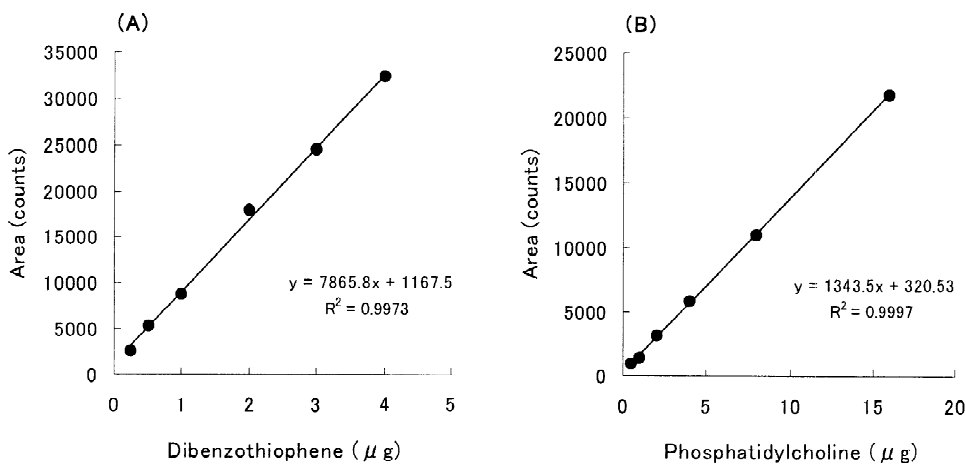

Fig. 5. Response curves for dibenzothiophene and phosphatidylcholine. (A) Response curve for dibenzothiophene; (B) response curve for phosphatidylcholine.

reported that the relationship between the intensity of the S_2 related emission and the amount of sulfur compound was given by following equation:

$$i_E = i_0 (M)^n$$

where i_E is the intensity of the emission, M is the amount of sulfur, and i_0 and n are constants for given experimental flame conditions. The n varies between 1.69 and 2.0 depending on the flame condition. Brody and Chaney [22] noted that the response to sulfur compounds varies exponentially and that the FPD response is equal to the square of the concentration $[S]$ of sulfur, i.e. Response $= [S]^2$. The result of (A) in Fig. 5 is different from previous studies. In this study, a Chromarod occurs within the active zone of the hydrogen flame and this factor, typical for Iatroscan TLC–FID application, is very different to the conditions of ion formation in the FID or FPD of GC detector. As an example of this, Karlsen and Larter [6] observed that sulfur containing compounds give a higher per gram response in the Iatroscan FID as compared to hydrocarbons. This behavior is quite opposite to normal GC–FID response where the heteroatoms are seen as ''diluting'' the FID response due to carbon. It is therefore quite clear that introducing the Chromarod and the high hydrogen flow in the Iatroscan affects ion formation; this most likely also applies to FPD. The mechanism of S_2 emission is the competitive reaction [26], and the result suggested that components such as SiO_2 of the Chromarod affect the formation of S_2 species from sulfur atom and/or the stage of excitation reaction to S_2^* as a catalyst.

However, the response curve of PC due to HPO emission was found to be linearly proportional to the amount of PC, and this result is consistent with previous studies.

3.4. Analysis of samples containing sulfur and phosphorus

The selective detection of sulfur and phosphorus compounds in heavy oils and human serum lipids demonstrates the utility of the TLC–FID/FPD system; the chromatograms are shown in Figs. 6 and 7. The resulting thin-layer chromatograms of FID and
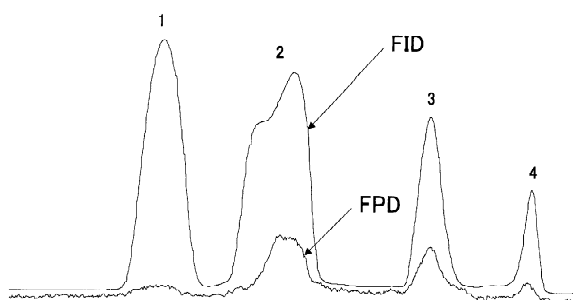

Fig. 6. Chromatogram of heavy oil with TLC–FID/FPD system in the sulfur mode. 1: Saturated hydrocarbons, 2: aromatic hydrocarbons, 3: resins, 4: asphaltenes. Sulfur compounds are found mainly in the aromatic fraction as benzothiophenes and in the high molecular mass resin and asphaltene fractions.

FPD for the heavy oil sample are shown in Fig. 6, and distribution of sulfur compounds in the heavy oil can be discerned. Note that DT elutes together with the polyaromatic hydrocarbons. The chromatograms of human serum lipids are shown in Fig. 7. Each fraction of phospholipids in the extracted lipids is identified in the FPD responses.

4. Conclusion

The TLC–FID/FPD system is capable of detecting sulfur and phosphorus in materials with a high boiling point, such as heavy oils and phospholipids. Molecular emission spectra due to the S_2 and HPO
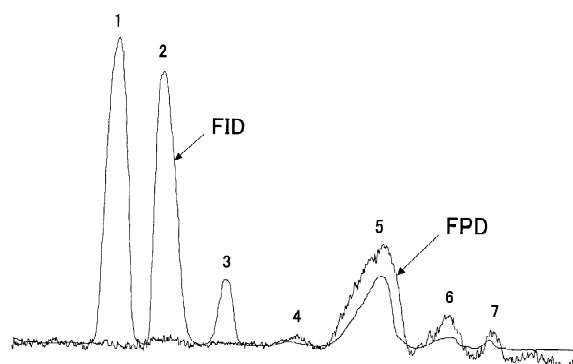

Fig. 7. Chromatogram of human serum lipids with TLC–FID/FPD system in the phosphorus mode. 1: Cholesterol ester, 2: triglyceride, 3: cholesterol, 4: phosphatidylethanolamine, 5: phosphatidylcholine, 6: sphingomyelin, 7: lysophosphatidylcholine.

species from sulfur and phosphorus compounds were observed, and 394- and 526-nm interference filters were chosen for the TLC–FID/FPD system. Air flow-rate and scan speed affected the FPD response. An air flow-rate of 500 ml/min and a scan speed of 30 s/rod coupled with a hydrogen flow-rate of 160 ml/min gave optimum FPD response for DT. An air flow-rate of 1500 ml/min and a scan speed of 40 s/rod, coupled with a hydrogen flow-rate of 160 ml/min gave a good FPD responses for PC. However, the strong interaction between PC and the silica gel of the Chromarod presumably affected the process of light emission. Therefore, the detection conditions, especially for high polar compounds, should be scrutinized and investigated in detail before analysis. Both DT and PC were observed to yield linear response curves within the investigated mass ranges of 0.25–4 and 0.25–16 μg, respectively, and this result is different from that observed for GC interfaced FPD systems.

Acknowledgements

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