APPLICATION OF THIN-LAYER CHROMATOGRAPHY WITH FLAME IONIZATION DETECTION TO THE CHARACTERIZATION OF ORGANIC EXTRACTS FROM DIESEL EXHAUST PARTICULATES

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SUMMARY

Thin-layer chromatography with flame ionization detection has been applied to the analysis of organic extracts of diesel exhaust particulates. Non-volatile organics whose boiling points are higher than *ca.* 300°C can be analyzed, with a detection limit of *ca.* 0.03 µg and a relative standard deviation of *ca.* 10%. The organic extracts are separated into aliphatics, aromatics and polars by the development procedure with *n*-hexane, and the polars can be separated further by using toluene as the solvent. Some examples of application are presented.

INTRODUCTION

There is currently worldwide concern over the health effects of diesel exhaust particulates which are thought to be one cause of chronic lung disease, and a number of extractable organic substances contained in diesel exhaust have been found to have mutagenic or carcinogenic activity[1]. The chemical characterization of these extractable organics has been performed by a number of methods and the extent of the separation has ranged from simply weighing the total amount of the extracts[2] to the individual identification such as benzo[*a*]pyrene by high-performance liquid chromatography[3]. The selection of the analysis procedure should be done according to the purpose of the study because, generally, as the separation efficiency rises, overall quantification of the organic extracts becomes difficult.

We have been studying the relation between the fuel properties and the exhaust emission from diesel engines and are concerned primarily with the overall toxicity of the exhaust. For this purpose, the identification and quantification of individual chemical species may not be necessary and can be difficult, considering the number of species and the ambiguity in determining the toxicity of carcinogenicity of each substance. However, measurement of the weights of the extractable organics alone is not enough to estimate the toxicity of particulates because their biological activity has been found to vary with the type of the organics (classified into aliphatics, aromatics, moderately polar neutrals, highly polar neutrals, bases and acids[4]). The Ames

bioassay for mutagenicity indicated that moderately polar neutral compounds and the aromatics have high mutagenic activity. The method of separation into classes as above was based mainly on liquid–liquid extractions and column chromatography. Although this method is simple in principle, it seems to be time- and solvent-consuming, has low sensitivity and also takes great skill to perform.

Thin-layer chromatography (TLC) with a flame ionization detector has been succesfully applied to the analyses of thermolabile and polar organic compounds not suitable for gas chromatography[5,6]. In this paper we report the application of this method to the analysis of the organic extracts of diesel particulates. It enabled us to quantify 2–3 μg of the organic extracts according to three types: aliphatics, aromatics and polars.

EXPERIMENTAL

The equipment used for TLC was the Iatroscan TH-10 (Dia-Iatron Co.). The recording of chromatograms and the integration of the peaks were performed by a Chromatogram Processor 7000A (System Instruments Co.). For the solid phase of the chromatography, a quartz glass stick (0.9 × 153 mm) coated with a Chromarod S II silica gel fused glass ceramic layer was used. Prior to use, the rod was activated by passing it through the flame of the detector and put in a constant-humidity chamber (58%) for 10 min in order to make constant the activity of the silica gel on the rod. Typically, 2 μl of dichloromethane extracts of diesel exhaust particulates were spotted near one end of the rod and were developed with *n*-hexane for about 25 min or about 10 cm in a development chamber. Then the rod was taken out of the chamber, air-dried for 2 min completely to remove the solvent and analyzed by the Iatroscan.

The diesel exhaust particulates examined were those emitted from a single-cylinder test engine for the measurement of the fuel cetane number (ASTM CFR/F5), which was operated at a compression ratio of 16:1, a fuel injection rate of 15 ml/min,
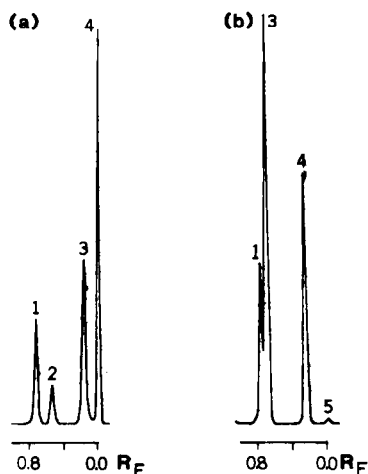

Fig. 1. Thin-layer chromatogram of text mixtures obtained with FID. Solvents: a, *n*-hexane, b, toluene. Peaks: 1 = *n*-eicosane; 2 = *n*-dodecylbenzene; 3 = benzo[*a*]pyrene; 4 = anthraquinone; 5 = impurities.

an air to fuel ratio of *ca.* 19:1 and a speed of 900 rpm. The exhaust gas from the engine was diluted about thirteen times in a dilution tunnel (Horiba Seisakujo Co.) and was passed through a silica thimble (Whatman, 25 × 90 mm, tapered), which had been pretreated with dichloromethane for 6 h to remove the background extractable organics. The amount of the particulates collected was estimated by weighing the thimble before and after the collection, and it was almost within 10–20 mg. The thimble was then extracted with 100 ml of dichloromethane in a Soxhlet apparatus for 18 h and the extract was concentrated to about 3 ml by distillation. Then, it was transferred to a 10-ml vial, and the solvent was evaporated in a nitrogen stream at ambient temperature. The amount of the total extracted residue was estimated from the difference between the weight of the vial with the residue and that of the empty one. The residue was re-dissolved with 1 ml dichloromethane, and 2 μl were applied to the Chromarod for the analysis described above.

The standard chemicals were analyzed in the form of 0.1–0.3% dichloromethane solutions. The dichloromethane used for the extraction was of high purity, commonly used for testing agricultural chemical residues (Wako Chemicals Co.) and was distilled once to remove non-volatile contaminants.

RESULTS AND DISCUSSION

Separation of the organics on the Chromarod

Fig. 1a shows the result of TLC of a standard mixture of *n*-eicosane, *n*-dodecylbenzene, benzo[*a*]pyrene and anthraquinone, with *n*-hexane as solvent. The R_F (rate of flow) values of the aromatics ranged from 0.11 for benzo[*g,h,i*]perylene to 0.50 for dodecylbenzene. On the other hand, normal paraffins exhibited almost the same R_F value (0.70) regardless of the length of the carbon chain. Polar compounds, having at least one heteroatom, remained at the start point, that is, their R_F values were almost zero. These values are almost the same as those on a normal thin-layer[7]. One of the advantages of this method is that the polar compounds, which hardly move, can be examined by flame ionization detection (FID). As above, by using *n*-hexane as the solvent, the extracts can be classified into three types: aliphatics, aromatics and polars. The separation is poorer compared with liquid–liquid separation combined with column chromatography[4], especially for the polars, since with the latter method these compounds are further separated into moderately neutrals, highly polars, acids and bases. However, this TLC procedure is much simpler, because it does not need any pre-treatment other than Soxhlet extraction and concentration. In addition, it is possible to separate the polars further by using toluene as the solvent (Fig. 1b), since in this case the R_F value of carbazole is 0.64, that of anthraquinone is 0.21, while the R_F values of higher polars, such as acids and bases, are still almost zero. The R_F values of several substances in various solvents are presented in Table I.

Quantification of each type

The advantage of FID lies in its selectivity and high sensitivity towards carbon, namely towards organics, and the ease of quantification of hydrocarbons since the sensitivities of all hydrocarbons towards FID are of the same order. In Fig. 2 the sensitivities of several normal paraffins and polycyclic aromatic hydrocarbons for the

TABLE I

R_F VALUES OF SEVERAL SUBSTANCES DEVELOPED IN VARIOUS SOLVENTS

Solid phase = Chromarod S II.

Solvent	Eicosane	Dodecyl-benzene	Phenan-threne	Benzo [a]pyrene	Carbazole	Anthra-quinone
n-Hexane	0.67	0.52	0.25	0.14	0.02	0.00
Cyclohexane	0.67	–*	0.25	–	–	–
n-Hexane–toluene						
(2:1)	0.77	0.74	–	0.56	–	0.03
Toluene	0.79	0.79	0.70	0.72	0.64	0.21
Dichloromethane	0.82	0.85	–	0.79	0.77	0.69
Tetrahydrofuran	0.79	–	–	0.74	0.77	0.74
Acetone	0.79	0.82	–	0.74	0.77	0.72
Methanol	0.77	0.79	–	0.69	0.74	0.03

  * Not analyzed.

Iatroscan are shown, with the horizontal axis representing the carbon number in each molecule. Unlike the case of the usual gas chromatography with FID, the sensitivity decreases sharply for substances whose boiling points are below *ca.* 300°C and becomes zero for *n*-dodecane (b.p. = 215°C). Two kinds of evaporation of the sample are thought to be the cause of this effect: evaporation just in front of the FID flame and that during the spotting and development. The former, particularly, is thought to have a big effect, in which the sample evaporates due to the heat of the FID flame before entering the FID flame zone and is not detected. It is concluded that quantification with this method is possible for organic substances whose boiling points are above *ca.* 300°C.

The reproducibility of the intensity is presented in Table II, with *n*-eicosane, chrysene and anthraquinone representing the aliphatics, aromatics and the polars,
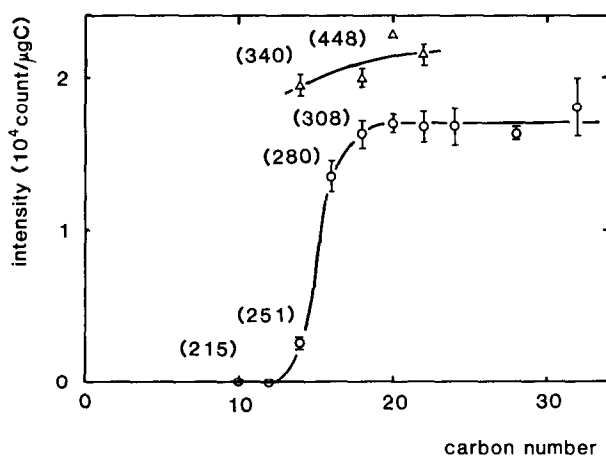

Fig. 2. Sensitivities of FID to normal paraffins (O) and polycyclic aromatic hydrocarbons (PAH) (△). Numbers in parentheses represent the boiling points (°C) of the compounds.

TABLE II

REPRODUCIBILITY OF THE INTENSITY OF THE TLC CHROMATOGRAM WITH FID

S.D. = Standard deviation (no. of samples 20), C.V. = coefficient of variation. Solid phase = Chromarod S II; mobile phase = n-hexane.

	Eicosane (0.480 μg)	Chrysene (1.105 μg)	Anthraquinone (0.635 μg)
Mean*	9503	23,654	19,260
S.D.*	1029	1482	2101
C.V. (%)	10.8	6.3	10.9

    * Unit is "count" of the integrator.

respectively. The relative standard deviation was about 10%. With this apparatus, ten Chromarods can be simultaneously treated, so that in order to keep the deviation within *ca.* 5%, five Chromarods were used for the analysis of one sample in later analyses. The intensity was not influenced by the spot size within the range from 2 to 5 mm.

    The relationship between the amount of benzo[*g,h,i*]perylene and the intensity of the chromatogram is shown in Fig. 3. With amounts from 0.08 to 3 μg, the exponent of this calibration curve was 1.22, somewhat larger than unity. In later analyses, however, the calibration was made at one concentration, since the range of the measured concentration was relatively narrow. Even if the substances are not volatile, the sensitivities differ slightly according to the type of species so that the calibration was made for every type. The detection limit was about 0.03 μg.

Application to the analyses of diesel exhaust particulates
    Fig. 4a shows the chromatogram of a dichloromethane extract of diesel exhaust particulates, with n-hexane used as the solvent. By comparing this with Fig. 1a, the
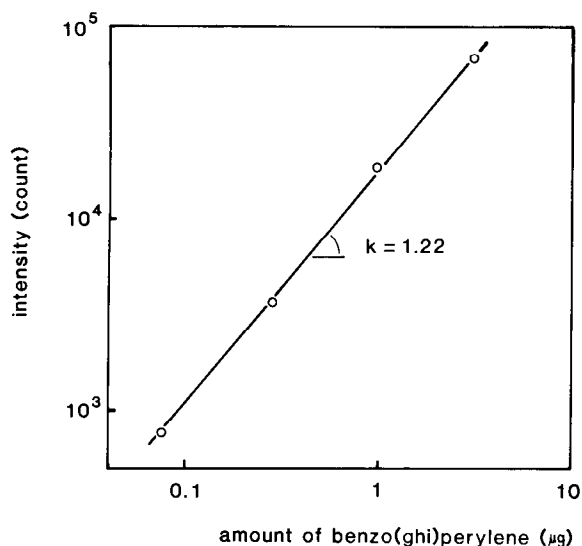

Fig. 3. Relationship between the amount of benzo[*g,h,i*]perylene and the intensity of the chromatogram.
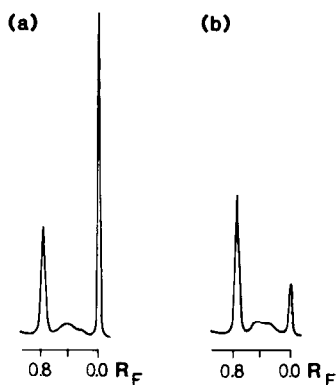
(a)   **(b)**

Fig. 4. Chromatograms of extracts of diesel exhaust particulates: a, dichloromethane extracts; b, *n*-hexane extracts. Mobile phase: *n*-hexane.

sharp peaks around $R_F = 0.7$ and 0.0 are attributed to the aliphatics and polars, respectively, and the broad peak from 0.1 to 0.5 to the aromatics. The aromatics could not be separated further, although a couple of peaks or shoulders can be observed in this broad peak.

As a first example of the application of this method, the results of the measurement of Soxhlet extraction rates are shown in Fig. 5 for each of the three types of compounds. The extraction solvent was dichloromethane. It is evident that the extraction is almost completed within 6 h for all the types.

The nature of the extracts varies with the extraction solvent and the change in the total amount of organic extracts was studied using an organic carbon analyzer[8]. With the TLC method reported here, changes in the chemical properties of the extracts can also be observed. Fig. 4b is the chromatogram of a *n*-hexane extract of diesel exhaust particulates. The total amount of the organic extracts decreased slightly as previously reported[8]. In addition, it was found that the aliphatic portion increased when using *n*-hexane as the solvent, as would be expected.

As mentioned in the Introduction, we are now studying the relationships between fuel properties and the emission of particulates from diesel engines employing this analytic method. Complete results will be reported elsewhere. An example is
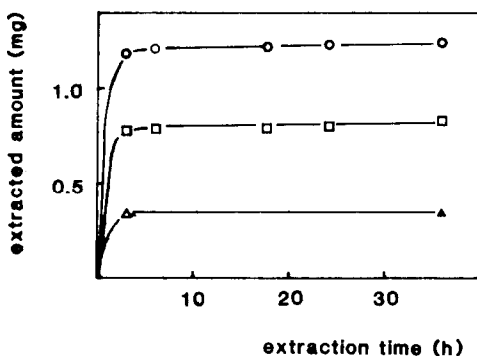

Fig. 5. Soxhlet extraction rates for the three types of extracts: O, aliphatics; △, aromatics; □, polars. Extraction solvent: dichloromethane. Amount of particulates extracted: 21.54 mg.
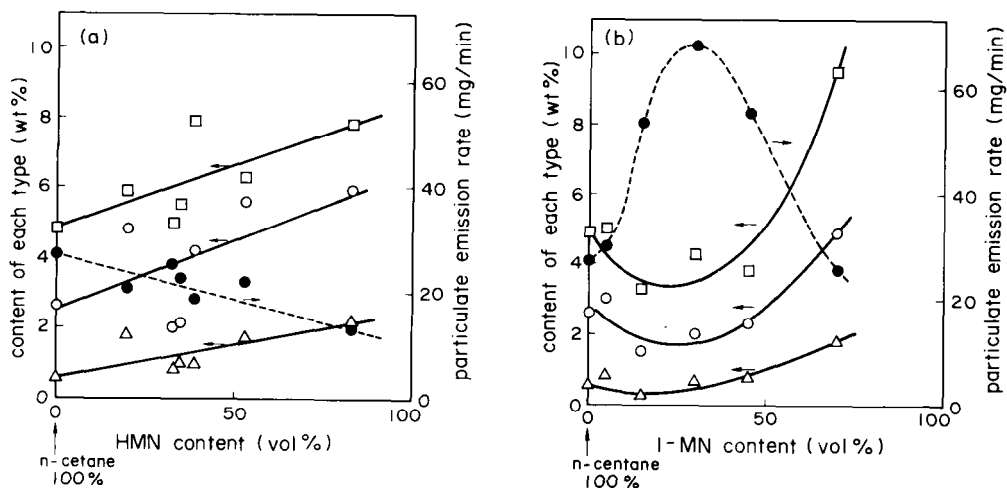
Fig. 6. Effect of the composition of diesel fuel on the particulate emission rate and the content of extracts of the particulates. Open symbols and solid lines indicate the content of extracts: ○, aliphatics; △, aromatics; □, polars. Filled circles and the broken line indicate particulate emission rates. Fuels: a, *n*-cetane + heptamethylnonane; b, *n*-cetane + 1-methylnaphthalene.

shown in Fig. 6a, which shows variations in the content of each type of extracts in the exhaust particulates with the composition of the fuel used in the diesel engine. The fuels were mixtures of *n*-cetane and heptamethylnonane (HMN), both of which are being used as standards of the cetane number by ASTM. This measurement was performed in order to find out whether the degree of branching in the aliphatic fuel has any effect on the emissions. As the content of HMN increased, the content of the organic extracts increased, while the amount of the particulates decreased, so that the change in the amount of the organic extracts, which is the product of the above
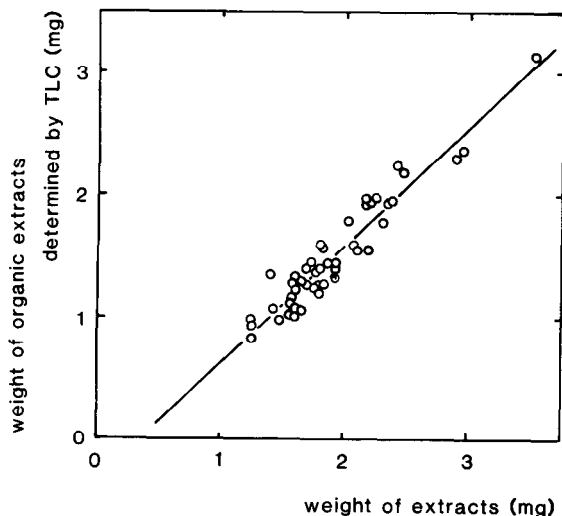

Fig. 7. Relationship between the total amount of organic extracts determined with the Iatroscan and the amount of the extracted residue measured by weighing.

two values, was small. Fig. 6b shows the result of a similar experiment except that the HMN was replaced by 1-methylnaphthalene (1-MN). In this experiment, the effect of the content of aromatic carbon in the fuel on the emission was studied. The contents of all the three types of organics were at a minimum when the content of 1-MN was around 25%, while the amount of the particulates emitted was maximum near this point. Again, the content of organics was negatively correlated with the amount of particulates. In both experiments, the change of the unburnt fuels being characterized as extractable organics is relatively small because the boiling points of *n*-cetane, HMN and 1-MN is 287, 240 and 240°C, respectively, namely below 300°C, and additionally, the particulates collected on the filter are put in a desiccator over night in order to remove volatile substances. Therefore, the aliphatics and the aromatics detected with this method are mostly the products of reaction between the fuels. The polars are thought to arise from partial oxidation of the fuels.

Fig. 7 shows the relationship between the total amount of organic extracts determined with the Iatroscan and the amount of extracted residue measured by weighing each collection of particulates. The two quantities are well correlated ($r = 0.954$). The fact that the slope of the line is nearly unity (0.97) indicates that the sensitivity factors were adequate. The existence of a few tenths of a milligram in organics which are intensitive to FID is inferred from the deviation of the line from the origin. At any rate, the high correlation coefficient indicates that this analytic method has high reliability for the quantification of organic extracts of diesel exhaust particulates.

CONCLUSIONS

Thin-layer chromatography with FID is a simple method to analyze organic extracts of diesel exhaust particulates. It permits those organics whose boiling points are higher than *ca.* 300°C to be classified into several types by a development procedure with *n*-hexane or toluene and quantified for every type without difficult pretreatment. The detection limit is *ca.* 0.03 μg and the relative standard deviation of the quantification is *ca.* 10%, which means that by taking the mean value of several measurements the relative error can be decreased to a few per cent. Some applications of this method were presented, one of which showed that the content of each type of extractable organics in diesel exhaust particulates varies with the fuel composition.

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