IATROSCAN INSTRUMENT APPLICATION

TLC/FID

No. 11

Optimising Reproducibility with the IATROSCAN TLC/FID Analyzer

Unless experimental conditions in TLC/FID analysis are constant, it may sometimes be difficult to ensure good reproducibility. The fingerprinting of a topped heavy oil can be taken as a typical example of such a case, and so a technique to optimise reproducibility will be presented.

Analytical Patterns of Heavy Oil by IATROSCAN TLC/FID

The fingerprinting of heavy oil can be made by TLC/FID much more rapidly and simply than by the classical column chromatography method. However, unless the analysis is undertaken with a full understanding of the characteristics of the thin layer chromatography and flame ionization detection, good reproducible data may sometimes be difficul to achieve.

1. Special features relating to the fingerprinting of heavy oil:

Since heavy oil comprises a complex mixture of various substances which are impossible to isolate, a compositional analysis technique is generally with several eluting solvents of varying polarities. In this case, the degree of separation depends upon, (a) the properties of the substance in the sample (b) the activity of the CHROMARODS and (c) the polarities of the development solvents.
Consequently, unless an analytical condition such as the activity of the CHROMAROD can be made constant, reproducibility of the data will not be obtained. Furthermore, it is particularly important to remember that an open column method such as thin layer chromatography is apt to be affected by atmospheric conditions.

2. Procedure to optimising reproducibility:

In order to obtain reproducible data, it is necessary to ensure the control of the following analytical conditions in TLC/FID.

2-1 Activity of CHROMAROD.

Activity adjustment of the CHROMAROD is made in two steps by the use of a constant humidity chamber and a suspended development technique.

a. Use of a constant humidity chamber.

When a CHROMAROD is left exposed to a laboratory atmosphere, the silica gel or alumina coating on the surface of the CHROMAROD absorbs moisture quickly to reach equilibrium with room humidity because both materials are strong water adsorbents. Therefore, a CHROMAROD will begin to absorb room moisture immediately after blank-scanning in the FID burner has taken place and will reach equilbrium with room humidity in 5 to 10 minutes after the samples have been spotted. Since the degree of water adsorpition has a great affect upon the activity of the CHROMAROD, which, as already stated, is influenced by room humidity, the degree of separation of the sample can differ accordingly.

A constant humidity chamber is now used to make the degree of water adsorption constant and fully independent of the random atmospheric conditions to which hitherto, the CHROMAROD could be exposed. A CHROMAROD onto which a sample has been spotted now requires to be preserved in a constant humidity chamber for a short while end, after reaching equilibrium with the humidity within the chamber, is transferred to the developing tank to maintain a constant degree of water adsorption.

b. Preparation of a constant Humidity Chamber.

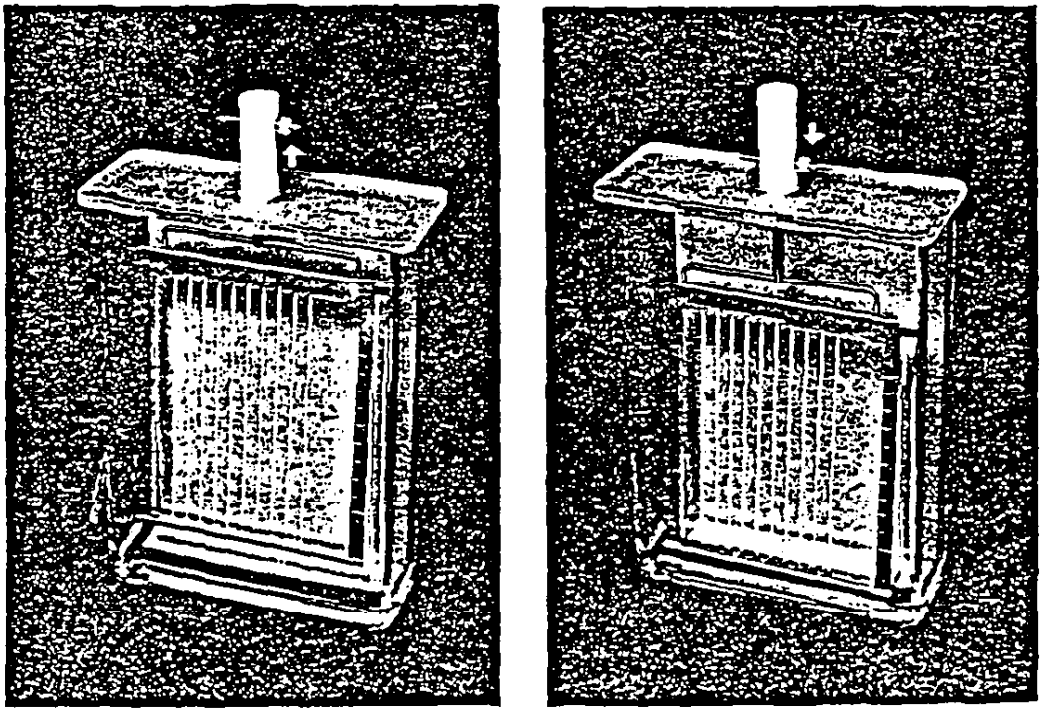
As an aqueous solution such as a sulfuric acide, saturated inorganic salt and others in a closed-type which show a constant humidity at a constant temperature, and these solutions can be used in a desiccator and/or developing chamber. Relationship between concentration of sulfuric acid and the relative humidity are shown in Table 1.

Table 1 Relative humidities and aqueous tentons of aqueous solutions H_2SO_4 at 25 °C.

% Humidity	Aqueous Tension	% H_2SO_4	% Humidity	Aqueous Tension	% H_2SO_4
100	23.756	0.00	50	11.88	43.10
95	22.57	11.02	45	10.69	45.41
90	21.38	17.91	40	9.50	47.71
85	20.19	22.88	35	8.31	50.04
80	19.00	26.79	30	7.13	52.45
75	17.82	30.14	25	5.94	55.01
70	16.63	33.09	20	4.75	57.76
65	15.44	35.80	15	3.56	60.80
60	14.25	38.35	10	2.38	64.45
55	13.07	40.75	5	1.19	69.44

Concentrations are expressed in percentage of anhydrous solute by weight.
Stokes and Robinson, *Ind. Eng. Chem.*, 41, 2013(1949).

C. Suspended Development Method (Preadsorption development method)



The suspended development technique ensures that the CHROMAROD is hung in the development tank for as long as it takes to absorb the necessary degree of solvent vapour before starting the Chromatographic development cycle. A chroma-rod suspended in the development tank gradually adsorbs solvent vapour to reach the required state of equilbrium in a given time. But if the suspending step is omitted, the degree of solvent adsorption degree on that part of CHROMAROD where the development solvent has not yet reached changes very moment as the time posses, as does the Chroma-rod activity, accordingly, reproducibility not satisfactory. On the other hand, when the rods are suspended in solvent vapours, no activity changes occur during development because the CHROMAROD is optimally pre-saturated and, thus reproduciibility is optimized.

Accessory equipment (see photo.) has been developed and manufactured by IATRON for exclusive use with the IATRO-SCAN TH-10 Mk-IV TLC/FID Analyzer and is specified in detail in this data sheet.

2-2 Quantity of sample and spotting method.

The quantity of sample spotted (absolute spotting weight) for each analysis should be prepared as accurately as possible. In this particular application, normally the sample is separated in 4 aliquots, saturated, aromatic, resin and asphaltene components, a total of 10 to 20mg/mℓ being spotted per CHROMAROD. The sample should not be broadly spread at the origin. It is essential therefore, not to spot 1μℓ in one application but to apply the sample little by little and with intermediate drying, using a low boiling point and low polarity solvent for dissoling the sample.

2-3 Development solvent and development distance.

These parameters should be closely controlled. Where combination of development solvents are used, take care to prevent decomposition and ratio changes due to volatization of the solvents. Fresh solvent should be prepared again when experiments are to be carried out next day.

2-4 Drying

Take care to prevent evaporation of the sample in the drying process after development. In cases where a volatile solvent such as hexane or toluene is used indoor air-drying will suffice provided it take place in a clean laboratory atmosphere.

2-5 Dispersion of sample on CHROMAROD

During the development of viscous materials the applied sample will gradually spread around the circumference of the CHROMAROD as the mobile phase takes the components along its length. Thus, a substance which has travelled the full length of the rod will have its components well dispersed around the sintered coating, which is the ideal position for them to be when FID scanning take place. However, if the development distance is over a short path length then the concentration of a viscous sample may tend to stay on the same side of the rod on which the spotting took place. In the case where components do not move from the origin then the position of the applied material is likely to remain more on that side of the rod and to spread around it only to a very limited extent. The extent to which the crosswise dispersion of the component concentration has occurred on the CHRCMAROD may cause the sensitivity of the FID to vary according to the location of the sample relative to the fixed position of the H_2 burner flame. In the case of the fingerprinting of heavy oils, and similar viscous substances, it is now recommended that immediately before FID scanning the CHROMARODS be turned 180° in the Rod Holder so that the side on which the sample was first applied is re-orientated face-down towards the H_2 burner.

3 Experimental results.

An example ot the fingerprinting heavy oil by the mentioned technique for optimising reproducibility is given below.

3-1 Experimental method

① Preparation of sample (10mg/mℓ dichloromethane solution).
② CHROMAROD-SIII - Blank scan to remove contaminants.

③	Sample spotting - 1μℓ in aliquots	
④	Preservation in constant humidity chamber - with sulfuric acid aqueous solution placed and relative humidity 65%	10 min.
⑤	Suspension in the first stage development tank	10 min.
⑥	First stage development (hexane)	10 cm.
⑦	Drying (solvent removal) at room temp.	2 min.
⑧	Preservation in constant humidity chamber, relative humidity 65%	10 min.
⑨	Suspension in the scond stage development tank	10 min.
⑩	Second stage development (toluene)	5 cm.
⑪	Drying (solvent removal) at room temp.	2 min.
⑫	Preservation in constant humidity chamber - relative humidity 65%	10 min.
⑬	Suspension in the third stage development tank	10 min.
⑭	Third stage development (dichloromethane : methanol 95 : 5)	2 cm.
⑮	Drying (solvent removal) at room temp.	2 min.
⑯	Scanning (orientate the spotting position face-down towards the burner)	

3-2 Experimental results.

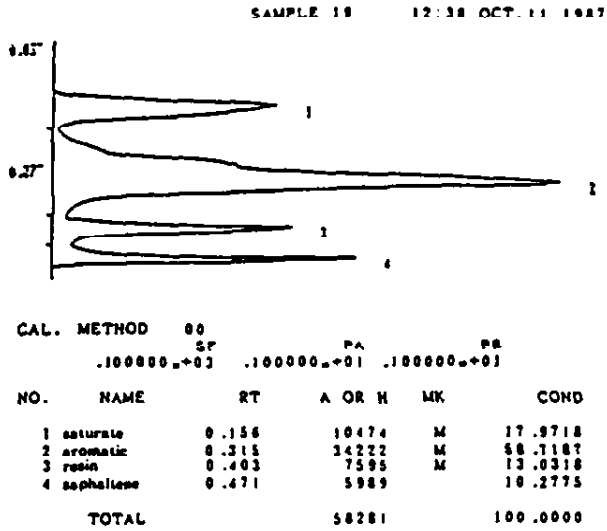
Sample : Topped residual oil

Separation pattern : refer to Fig. 1.

The mean value of ten (10) CHROMARODS-SIII was taken as the measurement result, and the reproducibility of daily error was calculated from the sample are percentage (as shown in Fig. 2 and Table 2).

When the spot changed in 5 to 30μg and measured under atmospheric residue, the calibration curves are shown in Fig. 3.

Fig 1 Topped residual oil



CONDITIONS :

Stationary phase : CHROMAROD-SⅢ

Mobile phase :

1st. Hexane 100% 10cm

2nd. Toluene 100% 5cm

3rd. Dichloromethane : Methanol

95 : 5 2cm

Gas flow : H_2 160ml/min, Air 2 l/min

Scanning speed : 30sec/scan

Integrator attnuation : 16

Table 2 Measured values of 10 CHROMARODS

CHROMAROD No.	Peak 1 Saturated	Peak 2 Aromatic	Peak 3 Resin	Peak 4 Asphaltene
1	17.6 %	58.9 %	13.4 %	9.9 %
2	18.1	58.5	13.2	10.2
3	17.8	59.3	13.0	9.9
4	18.4	59.3	12.8	9.5
5	18.2	57.7	13.4	10.7
6	18.5	57.9	13.0	10.6
7	18.3	58.8	12.4	10.4
8	18.0	58.7	13.0	10.3
9	18.2	58.6	12.6	10.6
10	18.8	58.9	12.6	9.7
$\bar{X}$	18.2	58.7	12.9	10.2
SD	0.34	0.53	0.34	0.41
CV %	1.9	0.9	2.6	4.0

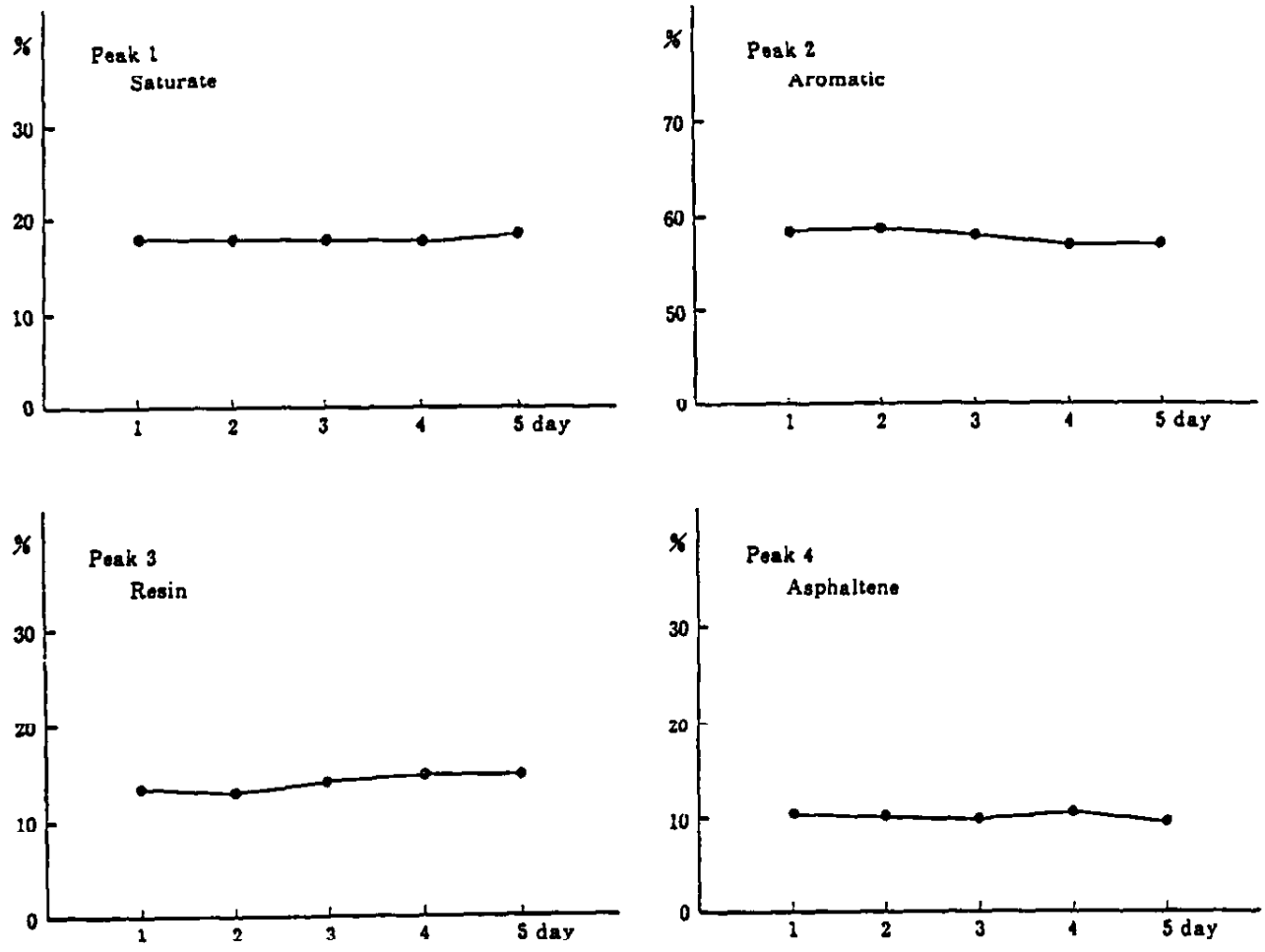


Fig. 2 Reproducibility of daily error

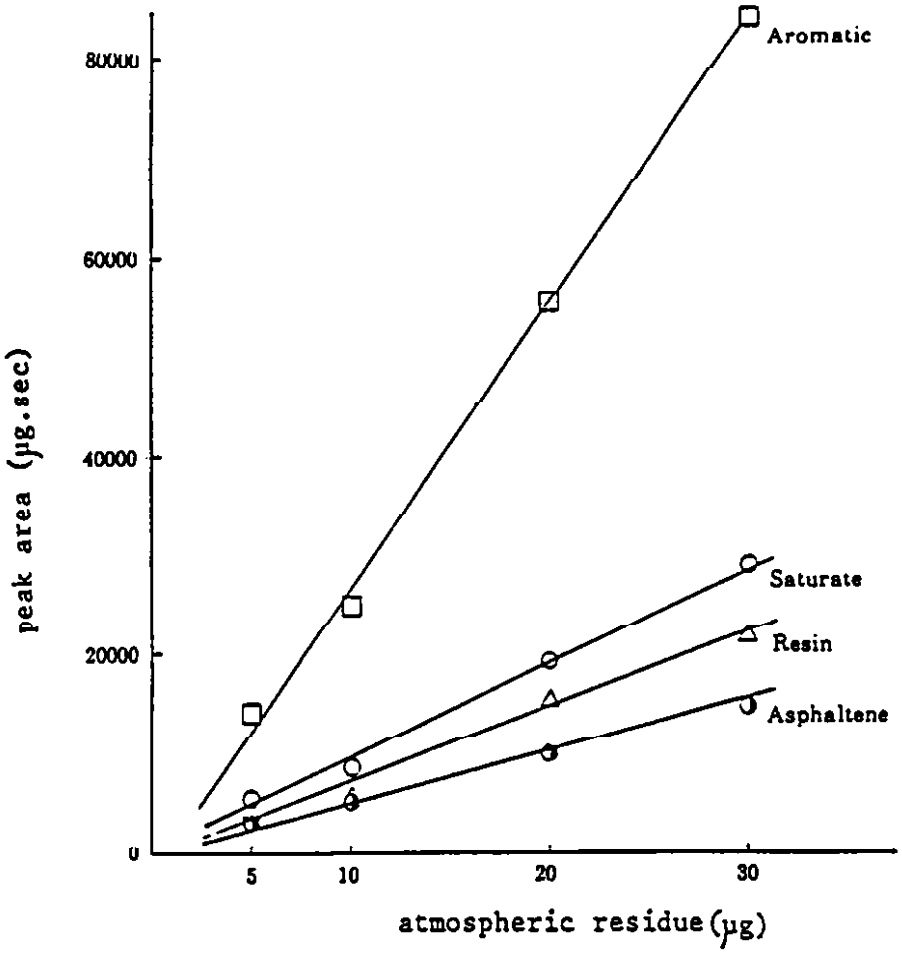


Fig. 3 Relationships between amounts of spot and peak area.

4. Conclusion

It is important that the various operating parameters of each analysis be kept constant in order to achieve reproducible data in the fingerprinting of heavy oil.

This statement applies not only to the fingerprinting of heavy oil, but to all types IATROSCAN analyses. Careful observation and practice with respect to these matters is essential in the case of quantitive measurements.