

Note

An improved method for the micro-separation of straight chain and branched/cyclic alkanes: Urea inclusion paper layer chromatography

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Abstract

We report a convenient paper layer chromatographic method involving urea inclusion for the separation of straight chain and branched/cyclic alkanes in saturated fractions from sedimentary organic matter. Compared with the well-known 5 A molecular sieving and traditional urea adduction methods, urea paper layer chromatography is more effective, simple and economic.

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1. Introduction

Separation of straight chain and branched/cyclic alkanes in saturated fractions from sedimentary organic matter is routine. A “clean” straight chain or branched/cyclic fraction is necessary for compound-specific stable carbon and hydrogen isotopic measurements and biomarker investigations; 5 A or ZSM-5 molecular sieving and traditional urea adduction are commonly used, but are not ideal methods (Murphy, 1969; Hoering and Freeman, 1984; West et al., 1990). The molecular sieving procedure requires the use of hydrofluoric acid (HF), which is hazardous, to recover the *n*-alkanes from the sieve, whereas with traditional urea adduction the straight chain fraction usually contains branched/cyclic compounds in small but significant amounts and vice

versa. Furthermore, these methods usually need a certain amount of starting material (ca. 1–2 mg). This is rarely conveniently available for recent sedimentary samples, environmental samples and high maturity source rocks. In this paper, we describe a convenient paper layer chromatographic method involving urea inclusion for the separation of straight chain and branched/cyclic alkanes in saturated fractions from sedimentary organic matter. Comparative experiments with traditional urea adduction were conducted so as to determine the effectiveness of the improved method.

2. Experimental

2.1. Sample preparation

Two oil samples were selected to test the urea inclusion paper layer chromatographic method. Sample No. 1 is a normal crude oil and No. 2 is a severely biodegraded crude oil. Asphaltenes were removed by

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precipitation with petroleum ether (30/60) followed by filtration. The de-asphalted oils were separated into saturate, aromatic and polar (NSO) fractions using column chromatography with a mixture of pre-activated silica gel and alumina (9:1, v/v) as stationary phase, with petroleum ether (30/60), benzene and ethanol as the eluting solvents, respectively. The saturated fractions were then ready for further separation by urea adduction.

2.2. Reagents and materials

The reagents included petroleum ether with a boiling point range of 30–60 °C, dichloromethane, methanol, ethanol, sulfuric acid and anhydrous sodium sulfate. All were analytical grade. Petroleum ether (30/60) was washed three times with concentrated sulfuric acid followed by water, dried over anhydrous sodium sulfate and redistilled. Re-crystallization from ethanol was used to purify the urea. Filter paper (Whatman #1) was Soxhlet-extracted with dichloromethane/methanol (3:1v/v) for 6 h.

2.3. Procedure for urea inclusion paper layer chromatography

Filter paper was cut into rectangular pieces 6 cm high. The width varied from 1 to 20 cm according to the sample load (ca. 1 mg–2 mg/cm). The saturated fraction in petroleum ether (30/60) was applied to the paper as a band using a micro-scale syringe (the centre of the sample band was 1.6 cm from the bottom and the band was separated from the bottom, Fig. 1). The urea-saturated methanol solution was applied to the filter paper to cover the sample. After standing for 10 min or more

for complete adduction, the urea-impregnated paper was put into a thin layer chromatography tank and developed with petroleum ether (30/60). When the solvent reached the top of the paper, the latter was removed and a ca. 1 cm strip, containing the branched/cyclic alkanes, was cut off from the top and put into a Pasteur pipette. An oily band could usually be observed on the strip. The branched/cyclic alkane fraction was removed from the strip with petroleum ether (30/60). The straight chain alkane fraction was obtained by an additional two developments of the remaining paper. The first was with water till the urea moved up away from the sample band. The paper was dried in a desiccator containing silica gel and a second development with petroleum ether (30/60) was carried out, but in the reversed direction (sample end upward). When the solvent reached the top, the paper was removed and a strip, representing the top ca. 1 cm, containing the *n*-alkane fraction, was cut off and rinsed with petroleum ether (30/60).

Normally, the amount of urea used is at least five times that of the sample. A typical urea paper with sample load is depicted in Fig. 1. The whole process was conducted at a room temperature of 20–25 °C.

2.4. Traditional urea adduction

Briefly, a saturated fraction was added to a urea-saturated methanol solution and allowed to stand for 24 h at room temperature for complete adduction. The urea crystals were rinsed several times with cold petroleum ether (30/60) and dried under a nitrogen stream. The straight chain alkanes were recovered by dissolving the crystals with distilled water and extracting with petroleum ether (30/60). The branched/cyclic alkane fraction was obtained by removing the methanol with a nitrogen stream and extracting with petroleum ether (30/60).

2.5. Gas chromatography (GC)

The hydrocarbon fractions were analyzed using GC with a Hewlett–Packard 6890 gas chromatograph equipped with a 30 m × 0.32 mm i.d. fused silica capillary column coated with a 0.25 μm film of CP-Sil 8 CB. The temperature started at 80 °C, was held isothermally for 2 min and was then increased to 290 °C at 4 °C/min, followed by a 25 min hold at 290 °C. The carrier gas was nitrogen at a flow rate of 1.0 ml/min.

3. Results and discussion

Oils No. 1 and No. 2 (Figs. 2a and b) were separated into straight chain and branched/cyclic alkane fractions using urea paper layer chromatography and traditional urea adduction. As shown in Fig. 2a1 and a4, both methods could give a clean separation for sample No.

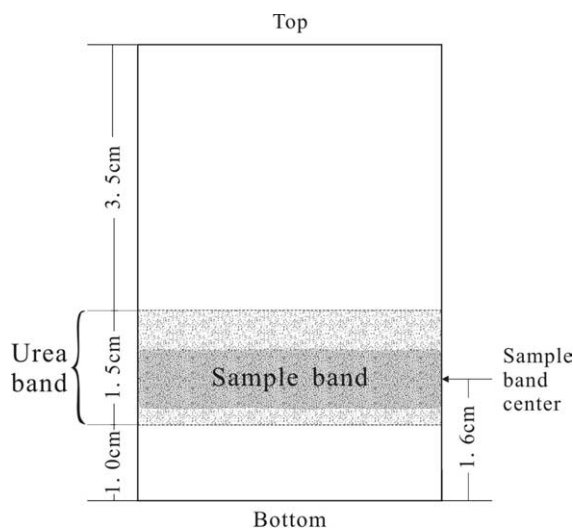


Fig. 1. Sketch showing urea inclusion paper layer chromatography.

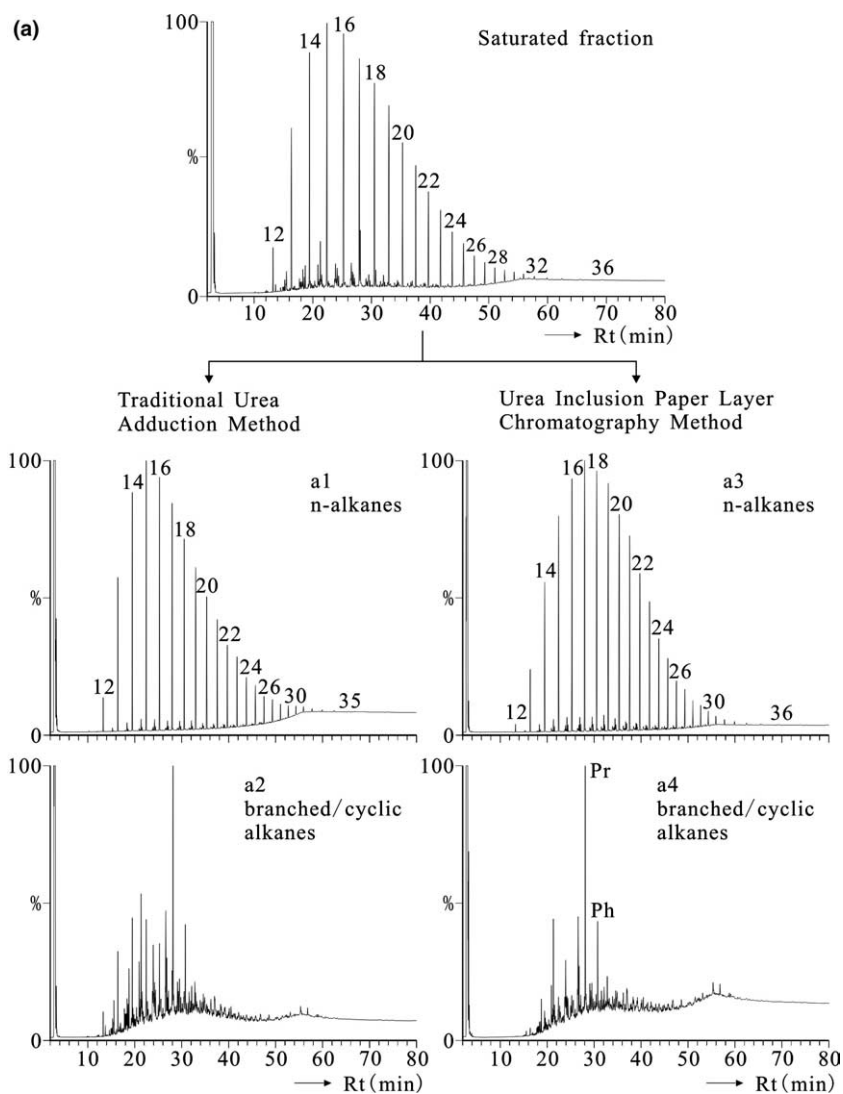


Fig. 2. Gas chromatograms of saturated hydrocarbons and sub-fractions using traditional urea adduction and urea inclusion paper layer chromatography for normal crude oil No. 1 (a) and severely biodegraded crude oil No. 2 (b).

1. GC/MS analysis found, however, that a significant amount of straight chain alkanes ranging from C_{12} to C_{20} were present in the branched/cyclic alkane fraction when traditional urea adduction was used (not shown). Theoretically, urea inclusion can provide an ideal separation of straight chain alkanes from branched/cyclic alkanes and the inclusion process itself is highly selective and complete. Therefore, work-up of the corresponding fractions during the experimental process possibly accounted for the incomplete separation. For example, incomplete washing of the inclusion solid would leave some branched/cyclic alkanes in the straight chain fraction. Over washing would partially destroy some crystals and make the branched fraction impure. Hence, it is usually recommended that the adduction should be car-

ried out 2 or 3 times to ensure complete separation. However, this makes it more time consuming and less convenient, even if it could be as effective if carried out 3 times. Either way, this is not the case for urea inclusion paper layer chromatography.

Sample No. 2, a highly biodegraded crude oil, is characterized by a very low concentration of *n*-alkanes and the presence of an unresolved complex mixture (Fig. 2b). As shown in Figs. 2b1 and b2, there was a poor separation with traditional urea adduction due to the low concentration of *n*-alkanes and the “*n*-alkane” fraction distribution was almost the same as the original mixture. However, a relatively pure straight chain alkane fraction was still obtained using urea paper layer chromatography (Fig. 2(b3)), even though the sample

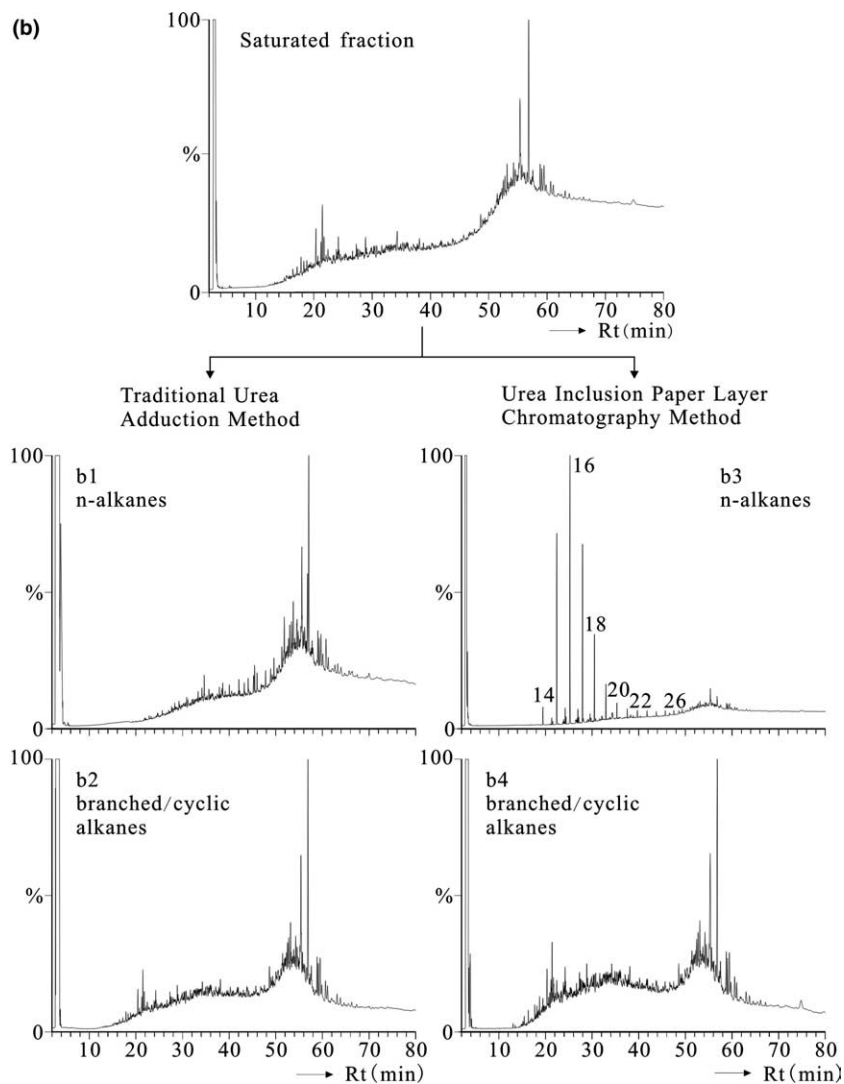


Fig. 2 (continued)

load was only one third (~ 10 mg) of that used in the traditional urea adduction procedure (~ 30 mg).

These two examples demonstrate that, by using filter paper as a support material, a chromatographic approach can be used and a clear separation of straight chain and branched/cyclic alkanes obtained. Furthermore, due to the affinity of filter paper for alkanes, the development with water only destroys the inclusion structure and moves the urea up and away from the sample band, leaving the straight chain alkanes almost exactly at the original location. The method omits the solvent extraction step in traditional urea adduction, which usually results in a low recovery of sub-fractions.

The whole process was carried out at a room temperature of 20–25 °C. We have noted that the separation was poor at temperatures below 15 °C, even when the fil-

ter paper was allowed to stand for 48 h before the first development. For 20–25 °C, 10 min standing are sufficient for complete adduction. This suggests that temperature could be an important factor in affecting the effectiveness of urea inclusion paper layer chromatography. Increasing temperature will, however, result in some loss of light components as shown in Fig. 2a3, where the main *n*-alkane peak has shifted two carbons upwards. Previous studies have shown that the inclusion compound crystals are typically several mm in length and a few tenths of a mm in cross-section and that the guest molecules must have a sufficiently long alkane chain, at least 7 carbon atoms, with only a limited degree of substitution (Okazaki and McDowell, 1984; Harris, 1993, 1996; Lee and Harris, 1999; Lann et al., 2000; Brussaard et al., 2002). Therefore, further investigations

are needed to minimize the loss of light components ($n\text{-C}_7$ to $n\text{-C}_{15}$) during the process of urea inclusion paper layer chromatography.

4. Conclusions

Traditional urea adduction tends to give fluctuating separation of straight chain and branched chain compounds and is time consuming. The urea paper layer chromatographic method is advanced in many aspects. It gives a clear separation of straight chain and branched/cyclic alkanes despite some loss of light alkanes due to the influence of temperature. A quick, tiny-scale separation is possible with this method. It is specifically useful for microanalysis of recent sedimentary samples, environmental samples, high maturity source rocks and severely biodegraded oils.

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