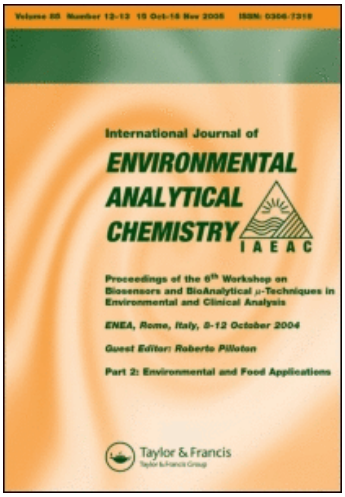

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Separation of Hydrocarbon Mixtures in the Microgram Range by Inclusion in a Urea-packed Mini Column†

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The use of classical urea inclusion techniques for the separation of straight chain hydrocarbons from branched and cyclic compounds is satisfactory when applied to mixtures in the milligram to gram ranges, but leads to low separation efficiencies when quantities in the microgram to milligram range are involved. In this study, a modified inclusion technique using an urea-packed milli-bore column and a catalytic eluent is described. Examples of its use for the separation of mixtures of linear and cyclic hydrocarbons from 30 μg up to a few milligrams are given. The versatility of this technique for the analyses of low amounts of environmental samples is described, and an application to the hydrocarbon fraction of surface sediment from lake Leman (Switzerland) is presented.

KEY WORDS: Urea inclusion, hydrocarbon separation, environmental samples, column chromatography, lake Leman.

INTRODUCTION

Since the discovery of the inclusion of organic compounds in urea channels,[1] many different aspects of this phenomenon have been

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investigated.[2,3] However, the most important practical application in this field remains the separation of straight chain or of some branched compounds from other branched or from cyclic compounds. This possibility has been extensively used in either industrial[2] (e.g. petrochemistry) or laboratory scale[3] (e.g. geochemistry, biochemistry).

Different techniques have been proposed for the inclusion of the adductable compounds. The most classical ones involve the urea-adduct formation by crystallization in a methanolic solution of urea.[2,3] Other techniques such as sublimation,[4] thin-layer chromatography[5] (TLC), column chromatography (CC)[6-9] and gas-solid chromatography[10,11] are also described. Nevertheless, among these various techniques only crystallization, TLC and CC have suitably been applied for group separations, but none of them has been mentioned to be useful in the submilligram range.

Since our laboratory is involved in a research project requiring detailed analyses of the hydrocarbon fractions from various environmental samples (air particulate matter, water, recent and ancient sediments and oils), and since very often only minute amounts of hydrocarbon (HC) mixtures are available (typically less than 1 mg), we were interested in a technique which could be used for mixtures in the microgram to milligram range.

To test whether the classical crystallization method could be adapted to this situation, experiments of separation have been done on standard hydrocarbon mixtures including several linear saturated hydrocarbons in the C_{11}–C_{33} range together with one cycloparaffin, i.e. cholestane. Briefly, this technique consists in dissolving the mixture in a urea non-adductable solvent (e.g. benzene, toluene) miscible with methanol, the HC/urea/methanol weight ratio being kept at 1:3:5. After complete dissolution, the mixture is refluxed, and, after cooling to room temperature and standing overnight at lower temperatures, the crystalline urea-adduct complexes formed are filtered; the filtrate contains the non-adductable compounds.

Without going into the details of these experiments, which have been conducted at different stand up times and temperatures, a satisfactory separation has never been obtained for mixtures of less than 5 mg. In fact, the success of the separation seemed to depend mainly on the total quantity of the HC mixture. Indeed, with a 50 mg HC sample, an inclusion yield of about 90% for the

adductable compounds and a recovery yield of 75–95% for the non-adductable ones could be achieved after three successive crystallizations. With 1 to 4 mg range mixtures, the best yields obtained were 65–85% and 40–60% respectively, the inclusion yield depending also on the sizes of the individual components. Moreover, the technique could not be used with samples containing less than 1 mg of HC. This prompted us to search for another inclusion technique.

Among the possible techniques mentioned in the first part, we decided to try the column chromatography (CC) technique which seemed to be the most versatile procedure applicable to minute HC mixtures. In fact, this method has been used for the separation of linear fatty acids from branched and cyclic ones in free[6,7] or esterified[8] fatty acids mixtures, but only in the 30 mg to 5 g range. In this paper we describe the adaption of the CC technique to the separation of HC mixtures in the submilligram range.

EXPERIMENTAL

Column preparation: Pyrex glass columns, 20 cm long and 1–2 mm internal diameter were made by flame stretching of commercial tubes of 1.5 cm i.d. At the top end, an unstretched length of about 10 cm was left to be used as solvent reservoir. The bottom end was filled with preextracted glass wool and the column was firmly packed with a weighted quantity of finely ground ($\geqq$180 mesh) recrystallized urea.

Procedure: Immediately after packing, the column was wetted with 500 μl of eluent. Three different solvent systems, with various amounts of samples were used in these experiments, i.e.:

—pure isooctane.
—isooctane-methanol: 100: 1–5 (w/w).
—isooctane-acetone: 100: 5–15 (w/w).

The HC mixture was dissolved in 100–200 μl of the eluent and deposited at the top of the column, followed by two portions of 500 μl of eluent in order to push the mixture into the urea. Then, 4 to 6 ml of solvent were added to wash out the non-adducted compounds. Different fractions of the eluent were collected to study the evolution of the separation.

The yields of recovery of the non-adducted compounds in the eluate were determined quantitatively in the initial mixture and in the eluates by mean of glass capillary-gas chromatography analyses performed with a Carlo-Erba FTV 4160 chromatograph equipped with an "on-column" Grob-type injector and a FID detector, and connected to a Hewlett-Packard 3388A integrator. The adduction yields were calculated either from the difference between the initial mixture and the eluate, or directly by dissolving the urea in water, and subsequently extracting the HC with methylene chloride and analysing it with GC.

The HC mixture was prepared from commercial compounds and was composed of C_{11}, C_{13}, C_{15},... C_{25}, C_{30} linear saturated paraffins and 5 α (H)-cholestane.

RESULTS AND DISCUSSION

In a first set of 15 experiments, isooctane-methanol solvent systems were used as eluents and various amounts of HC mixture (300 μg to 13.5 mg) combined to different HC mixture/urea ratios were tested; 13 of these tests did show no inclusion at all. The two others, which were both carried out with more than 5 mg of hydrocarbons, gave only partial separation. The result of the best one is reported in Table I. It is then obvious that the application of the CC technique, in a similar way but in a much smaller scale than those described for the fatty acids[6,7] and fatty acids methylesters,[8] is not possible for traces of hydrocarbons. This must be due to the difference in the quantities, and also probably to a more favorable adduction of compounds having a carbonyl functional group.[3,12,13,14]

In a second set of 20 experiments, pure isooctane was used as solvent, and again only two of them, both with more than 5 mg of hydrocarbons led to some separation. The results for the best of these two acceptable separations are also reported in Table I.

The use of a mixture of isooctane-acetone (100: 10; w/w) as solvent led actually to the expected separation. A set of 5 experiments using this mixture gave all positive results with various amounts of hydrocarbon samples, all of them below 1 mg, and with different urea/HC mixture ratios (1000 to 5000). The best inclusion yields were obtained with an urea/HC-mixture ratio greater than 2000.

TABLE I

Composition of the standard mixture and of the eluates giving the best results with (A) isooctane-methanol and (B) pure isooctane eluents. The quantities are normalized to cholestane = 100. Recovery of cholestane: A = 73%, B = 84%.

	n-C_{13}	n-C_{15}	n-C_{17}	n-C_{19}	n-C_{21}	n-C_{23}	n-C_{25}	Cholestane	n-C_{30}
Composition of initial mixture	4380	4090	4020	4520	3880	5870	5670	*100*	90
A Isooctane:MeOH (100:1; W/W)	7.1	37.9	51.9	52.6	46.6	44.7	39.4	*100*	26.8
B. Pure isooctane	22.9	70.8	83.8	66.8	32.1	23.5	18.4	*100*	13.1

Figure 1 shows the gas chromatograms obtained from a typical experiment with 300 μg of a standard HC mixture containing 91% of adductable components eluted from a column packed with 650 mg of urea. Adduction yields of individual linear HCs and recovery of cholestane are reported in Table II. These results show that, using 4 ml of eluent, 84% of the non adductable component is recovered in the eluate while more than 99% of the adductable C_{11}–C_{30} linear HCs are retained, except for n-heptadecane for which the adduction

TABLE II

Typical composition of the standard mixture (300 μg) and of the eluate (0–5 ml) using an isooctane-acetone mixture (normalization to cholestane = 100). Recovery of cholestane: 84%.

	n-C_{11}	n-C_{13}	n-C_{15}	n-C_{17}	n-C_{19}	n-C_{21}	n-C_{23}	n-C_{25}	Cholestane	n-C_{30}
Compositions of initial mixture	490	420	600	670	800	690	1130	1070	*100*	70
Composition in the eluate eluent: isooctane:acetone (100:10; W/W)	tr	1.3	5.9	34.7	11.7	tr	tr	tr	*100*	tr

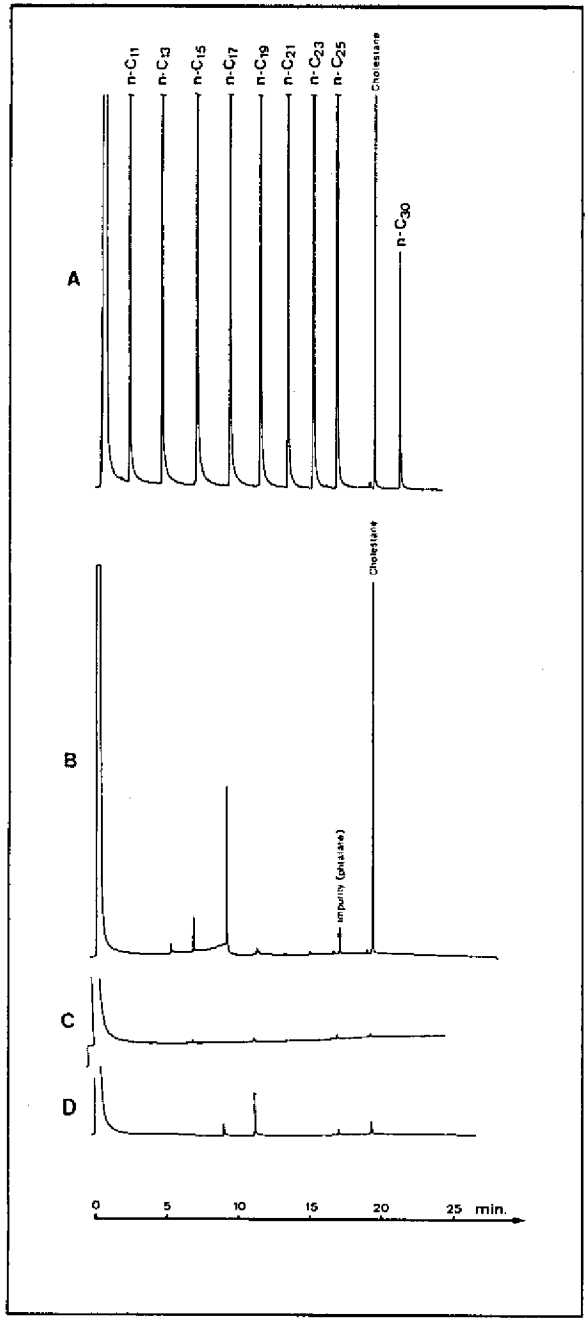

FIGURE 1 Gas chromatograms of the initial mixture (A) and those obtained after elution of 0–3 ml (B), 3–4 ml (C) and 4–5 ml (D) of the solvent. In (A) the peaks of n-alcanes are off scale (except for n-C_{30}).

yield is about 96%. The elution with larger volumes of solvent did not significantly improve the recovering yield of cholestane, while causing the release of small amounts of adducted compounds.

Figure 2 shows the results obtained when this technique was used with a complex HC mixture isolated from a surface sediment sample of lake Leman (Switzerland). A column containing approximately 600 mg of urea was used to separate the 80 μg of hydrocarbons present in this sample. The column was eluted with 9 ml of solvent and the fractions of 0–1.5; 1.5–4.5; 4.5–7 and 7–9 ml of the eluate were collected and analysed separately. The recovering yields of the major components eluting, on the GC, between n-C_{20} and n-C_{33} are reported in Table III. These results show again that in the first 4.5 ml of the eluate 78 to 96% of the non-adductable components are recovered while the linear HCs are almost quantitatively retained in the urea column. Subsequent fractions of the eluate show again little further recovery of the non-adductable compounds, together with some release of the adducted ones.

The choice of the isooctane-acetone mixture as eluent has been then the determining factor for the success of this method. In fact, the role of the solvent in the adduction processes with urea has been subject to some controversy. Briefly, the adduction is a two-phases process involving urea in the solid state. The role of the solvent is to bring the adductable compounds in intimate contact with crystalline urea. This is the case when pure isooctane is used as eluent. Nevertheless the addition of methanol to isooctane was thought to improve the rate of adduct formation, and this was generally interpreted by its action of dissolving and reprecipitating the urea in a finely divided form, more suitable for the interaction with the compounds to be adducted.[3,15] In the procedure we describe here we have added acetone to isooctane because of the catalytic role that some authors have attributed to this solvent as well as to other short-chain ketones.[2,4,16,17] Indeed, acetone forms with urea an unstable adduct with a half-life time of 4 minutes at room temperature,[2,12] and its catalytic action has been explained by the fact that the urea produced by the decomposition of such an unstable intermediate is more reactive towards the adductable HCs. The results presented in this paper confirm clearly this catalytical action of acetone and show that the addition of a urea dissolvent, like methanol, is not absolutely necessary.

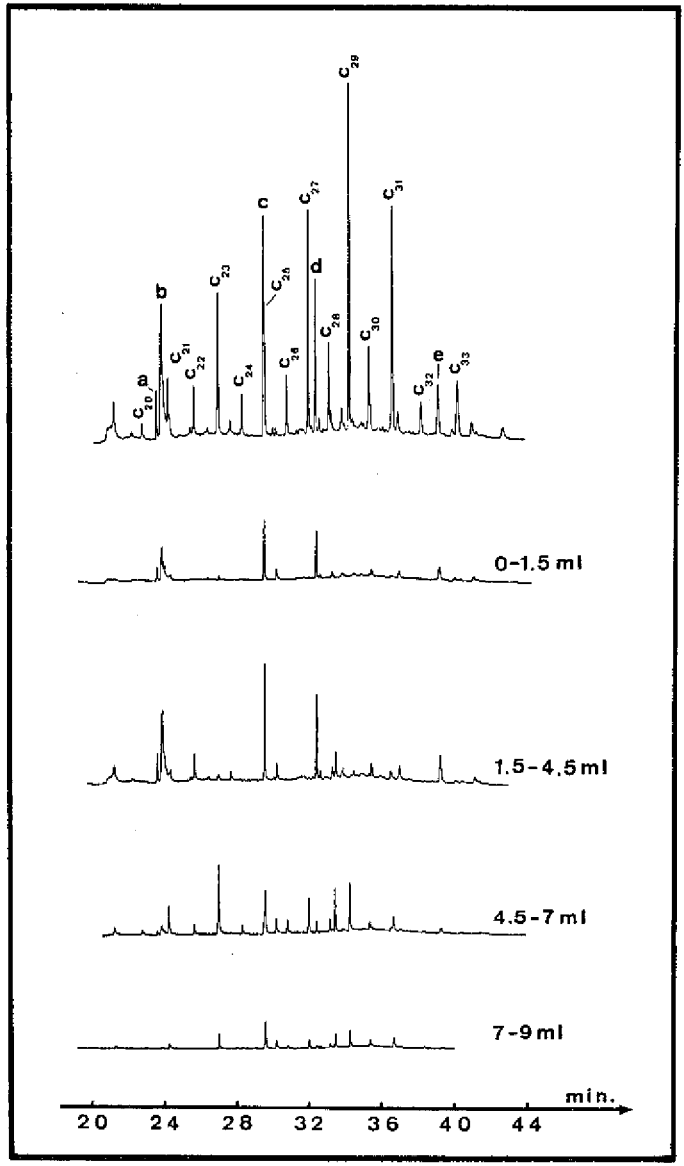

FIGURE 2 Gas chromatograms of the hydrocarbon fraction of surface sediment of lake Leman (top) and those of the different eluates. a, b, c, d, e: polycyclic hydrocarbons with 25(a, b), 29(e), 30(c) and 34(d) carbon atoms (given by GC/MS analyses). (b) is a doublet of isomers. The GC parameters and the dilutions were kept constant for all analyses.

TABLE III

Distribution of some compounds of a hydrocarbon mixture, obtained from a surface sediment of lake Leman (Switzerland), in the various fractions of the eluate from the urea column. The numbers are absolute values in arbitrary units of the areas of the GC peaks given by the integrator. The precision is ±5% due to syringe reproduction. In brackets: % of area relative to the initial value. a, b, c, d, e as in Fig. 2. The results for n-C_{21} and n-C_{28} are not reported due to errors in the calculation of the areas resulting from the partial coelution with vicinal peak.

Hydrocarbons / Fractions	a	b	n-C_{22}	n-C_{23}	n-C_{24}	C	n-C_{25}	n-C_{26}	n-C_{27}	d	n-C_{29}	n-C_{30}	n-C_{31}	n-C_{32}	e
Initial mixture	307	1835	370	1119	301	1474	825	447	1581	1012	2388	708	2042	371	618
A) Solvent elution 0–1.5 ml	87 (28.3)	567 (30.9)	—	—	—	423 (28.7)	—	—	—	328 (32.4)	tr	—	—	—	166 (26.9)
B) Solvent elution 1.5–4.5 ml	178 (58)	1202 (65.5)	207 (56)	tr	—	721 (48.9)	—	—	—	566 (55.9)	—	tr	93 (4.6)	—	403 (65.2)
A+B: Solvent elution 0–4.5 ml	265 (86.3)	1769 (96.4)	207 (56)	tr	—	1144 (77.6)	—	—	—	894 (88.3)	tr	tr	93 (4.6)	—	569 (92.1)
C) Solvent elution 4.5–7 ml	tr	163 (8.9)	109 (29.5)	626 (55.9)	77 (25.6)	201 (13.6)	284 (34.4)	130 (29.1)	290 (18.3)	79 (7.8)	349 (14.6)	98 (13.8)	197 (9.7)	tr	82 (13.3)
A+B+C: Solvent elution 0–7 ml	265 (86.3)	1932 (105.3)	316 (85.4)	626 (55.9)	77 (25.6)	1345 (91.3)	284 (34.4)	130 (29.1)	290 (18.3)	973 (96.2)	349 (14.6)	98 (13.8)	290 (14.2)	tr	651 (105.3)
D) Solvent elution 7–9 ml	—	—	—	281 (25.1)	—	tr	243 (29.5)	tr	74 (4.7)	—	115 (4.8)	69 (9.8)	115 (5.6)	—	—

CONCLUSION

Besides its simplicity, the technique of separation of the non-adductable compounds described here offers many advantages. As we have shown this procedure presents a high separation efficiency, since more than 80% of the adductable compounds are generally retained. Furthermore, the adducted components can easily be recovered by dissolution of the urea in hot water and subsequent liquid extraction with an organic solvent.

On the other hand the yield of the non-adducted compounds is over 80% in most cases. But the major advantage of this technique remains the extension of the application of urea inclusion down to 30 μg of hydrocarbon mixtures which allows its use for environmental studies.

However, even though very versatile, this technique still presents some drawbacks, particularly because the recovery yields cannot be increased without inducing a partial release of some of the adducted hydrocarbons (see Table III). Furthermore, the time necessary to carry out one separation is very long (up to 24 hours). Nevertheless it can be shortened by applying a weak nitrogen pressure on the column. Thus, with a 0.2 kg/cm^2 pressure, separation has been achieved in less than two hours, without loss of efficiency.

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