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Peak purity, yield and throughput in preparative liquid chromatography with self-displacement

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ABSTRACT

A cyclohexanone–cyclopentanone mixture was separated on silica with an average particle diameter of 5 or 25 μm using mobile phases of three different mixtures of hexane and *tert.*-butyl methyl ether. Two fractions were collected with the cut made at the lowest point between the two peaks. With increasing load the width of the first peak decreased, *i.e.*, the second compound began to displace the first. Under all conditions of high load the purity of the first fraction was markedly higher than that of the second; therefore, its recovery yield decreased because part of the compound was lost as an impurity in the second one. An equation for the calculation of the amount of collected fraction in milligrams is given; this amount depends on the purities of both fractions. Because the width of the fractions is known, it is then possible to calculate the yield, throughput and concentration of the fractions of interest, usually the first one. Of the three parameters purity, yield and throughput, it is possible to find conditions where two are kept high at the expense of the third by using a particular combination from the two stationary and three mobile phases.

INTRODUCTION

Preparative liquid chromatography, which is used to obtain pure compounds from a mixture, can be performed in various modes: analytical-scale separations with fraction collection, scaled-up elution chromatography under conditions of mass and/or volume overload, displacement chromatography [1] or frontal chromatography [2]. The frontal technique has not yet been investigated thoroughly but at least for special separation problems it could be useful. Displacement chromatography is gaining increasing interest because the throughpt is high; its drawback is the necessity to remove the displacer from the column after each separation. However, displacement effects are not uncommon in scaled-up elution chromatography if the mass overload is high enough. Under these circumstances the peak shapes of the individual compounds usually become triangular [3] (depending on the type of adsorption isotherm) and neighbouring peaks not only overlap but the later eluted compound can displace the less retained compound [4]. This phenomenon is not uncommon, as was shown recently [5,6], and can be used advantageously for the separation of two-component mixtures.

In this study, the behaviour of a mixture of two ketones when separated on silica under conditions of increasing mass overload was studied. Two columns of identical size but packed with stationary phases of 5 and 25 μm, thus giving different plate numbers, were used in combination with three mobile phases of differing elution strength.

EXPERIMENTAL

The columns used were stainless steel tubes of 25 cm × 10 mm I.D. When packed properly, such columns contain almost exactly 10 g of silica. Two batches of LiChrospher SI 100 (Merck, Darmstadt, Germany) were chosen by the manufacturer in order to use silicas with an as close matching of physical properties as possible: the 5-μm phase had a specific surface area of 393 $m^2 g^{-1}$ and a pore volume of 1.29 ml g^{-1}, whereas these values for the 25-μm phase were 406 $m^2 g^{-1}$ and 1.27 ml g^{-1}. Also, the nitrogen adsorption isotherms were almost identical. Packing of the columns was done in both instances by a

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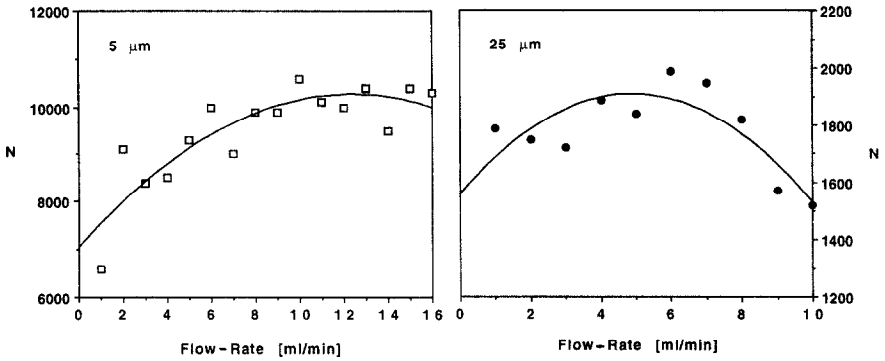

Fig. 1. Plate number, N, *vs.* flow-rate characteristics of the two columns used. Sample, 0.1 mg of cyclohexanone; mobile phase, hexane–*tert.*-butyl methyl ether (1:1). In both graphs a second-order polynomial is overlaid; however, for the 25-µm phase the maximum efficiency is at a higher flow-rate than the maximum of this curve. The data points are means of three measurements.

slurry method because no satisfactory results were obtained by dry packing of the coarse silica.

In Fig. 1, plots of plate number (N) *vs.* mobile phase flow-rate (kinds of van Deemter plots) of the two columns are shown. From previous studies it could be expected that under conditions of mass overload the column efficiency will be drastically lowered if the flow-rate is far from the Van Deemter optimum [7]. For the fine stationary phase no obvious optimum of the flow-rate (maximum N) was found and it was used at 14 ml min^{-1}. This corresponds to a reduced flow velocity v [8] of 4.4, where $v = (ud_p)/D_m$, u = volume flow-rate, d_p = particle diameter of the stationary phase and D_m = diffusion coefficient of the solute in the mobile phase, which can be calclated with the Wilke–Chang equation [9]. The value $v = 4.4$ is the reduced velocity calculated for cyclopentanone in hexane–*tert.*-butyl methyl ether (1:1). The coarse phase was used at 7 ml min, which is in the region of its optimum (see Fig. 1) and which corresponds to $v = 11$. Although the reduced velocities are not the same in both instances, the two columns are of equal quality if this is defined by the minimum reduced plate height, h [8], where $h = L/(Nd_p)$ and L = column length: $h_{5\,\mu m} = 5.0$ (with $N = 10\,000$) and $h_{25\,\mu m}$ 5.0 (with $N = 2000$). The plate height (and reduced plate height) is responsible for the purity of fractions and therefore for the yield, whereas the flow-rate (and reduced velocity) determines the throughput.

As mobile phase mixtures of hexane (hexane fraction) and *tert.*-butyl methyl ether, both of HPLC quality (Romil, Shepshed, UK), in volume ratios of 1:1, 3:1 and 9:1 were used.

The two-component mixture studied was cyclohexanone (first peak) and cyclopentanone (second peak), both of purum quality from Fluka (Buchs, Switzerland). These ketones have low UV absorptivity and therefore detector overload caused by too high a signal when large amounts of sample are injected is avoided; they also have almost identical molar absorptivities at the wavelength chosen (280 nm), which is advantageous when the shape of overlapping peaks has to be judged. They are separated with a low separation factor with the chromatographic system used here (if the separation factor were larger, separation by flash chromatography would be possible). They are cheap and non-toxic and can be analysed by gas chromatography. The sample was a 1:1 mixture (both by mass and by volume as the densities are identical) of the two ketones dissolved in the same volume of mobile phase (with the exception of "analytical" runs, where 0.1 mg of each was dissolved in 20 µl of mobile phase).

The following liquid chromatographic equipment was used: pump, Model 100 with preparative head (Altex, Berkeley, CA, USA); sampling valve, Model 7120 with various loop sizes (Rheodyne, Berkeley, CA, USA); detector, Uvikon LCD 725 at 280 nm (Kontron, Zürich, Switzerland); and recorder,

Tarkan 600 (W + W Electronic, Basle, Switzerland).

The touching and overlapping peaks were manually collected as two fractions. The fractionation was always made at the lowest point between the bands. For overlapping peaks this collection strategy is not backed by theoretical considerations but by the fact that the peak valley is the only criterion useful for simple laboratory-scale preparative separations. These samples were then analysed by capillary gas chromatography with the following equipment: stationary phase, OV-1701; column, Duran glass, 30 mm × 0.5 mm I.D.; film thickness, 1 μm; carrier gas, helium; flow-rate, 3.5 ml min^{-1}; chromatograph, Perkin-Elmer (Überlingen, Germany) Sigma 3; temperature programme, from 50 to 91°C at 2°C min^{-1}; injector, 250°C, splitless; detector, flame ionization, 250°C; and integrator, HP 3390 A (Hewlett-Packard, Palo Alto, CA, USA). Results were obtained by means of a calibration graph.

All data presented in the tables are means of two experiments.

RESULTS

Table I shows the behaviour of the chromatographic systems studied when used under analytical conditions, *i.e.*, without overload. The amount of sample is 0.1 mg of each of the ketones, giving a load of 0.01 mg of each compound per gram of silica. Capacity factor, k', plate number, N, and tailing, T (the ratio of the distances from peak maximum to peak end and peak front, respectively, measured at 10% of the peak height), are given for cyclohexanone (the first peak), whereas the sample mixture is represented by the separation factor α and the resolution R. It should be noted that the separation factor decreases with decreasing mobile phase polarity, *i.e.*, decreasing content of *tert.*-butyl methyl ether.

Tables II–VII present the results with increasing load for each of the two stationary and three mobile phases. Because the first fraction, from peak front to the valley between the peaks, is of higher purity than the second one, especially with increasing load, the calculations refer to the first compound. The question is always what purity, recovery yield and throughput of this substance can be obtained. The second fraction is of markedly lower purity and recycling would be necessary anyway if it was required to be obtained in pure form. It is clear from the results that conditions should be chosen in such a way that the compound of interest is eluted first.

By using the given sample mass and the experimentally found values of purities and fraction widths (in units of time or elution volume), it is possible to perform the calculations of interest, *i.e.*, to determine the yield as a percentage of the injected amount of cyclohexanone, the throughput in milligrams per minute and the concentration of the solute in the collected fraction in milligrams per millilitre.

The load per component in milligrams per gram is ten times lower than the mass injected in milligrams because the columns contain 10 g of stationary

TABLE I

CAPACITY FACTOR, PLATE NUMBER, TAILING, SEPARATION FACTOR, AND RESOLUTION OF THE DIFFERENT CHROMATOGRAPHIC SYSTEMS UNDER ANALYTICAL CONDITIONS

Columns: 25 cm × 10 mm I.D.; mobile phase flow-rate: 14 and 7 ml min^{-1} for the 5- and 25-μm phase, respectively. Ether is *tert.*-butyl methyl ether. Sample: 0.1 mg each of cyclohexanone and cyclopentanone in 20 μl of mobile phase.

Stationary phase (μm)	Mobile phase hexane–ether	k'_1	N_1	T_1	α	R
5	1:1	0.46	12 800	1.4	1.26	2.16
	3:1	0.76	13 000	1.6	1.21	2.50
	9:1	1.56	10 700	1.8	1.16	2.43
25	1:1	0.44	2390	2.2	1.26	0.86
	3:1	0.96	1860	2.2	1.20	0.98
	9:1	1.52	1840	2.3	1.16	1.00

TABLE II

PURITY OF FRACTIONS, AMOUNT OF FIRST FRACTION[a], RECOVERY YIELD OF FIRST COMPOUND[a], WIDTH OF BOTH FRACTIONS, THROUGHPUT OF FIRST COMPOUND[a], WIDTH OF FIRST FRACTION AND CONCENTRATION OF FIRST COMPOUND[a] WITH 5-μm STATIONARY PHASE, AND MOBILE PHASE HEXANE–*tert.*-BUTYL METHYL ETHER (1:1)

Chromatographic conditions as given in Table I with the exception of sample size.

Mass injected per compound (mg)	Load per compound (mg g^{-1})	Purity fraction 1 (%)	Purity fraction 2 (%)	Amount fraction 1 (mg)	Yield compound 1 (%)	Width both fractions (min)	Throughput compound 1 (mg min^{-1})	Width fraction 1 (ml)	Concentration compound 1 (mg ml^{-1})
0.1	0.01	100	100	0.1	100	0.32	0.31	1.63	0.061
1	0.1	99.3	96.5	0.971	97.1	0.325	2.99	1.63	0.61
3	0.3	98.9	95.4	2.89	96.3	0.367	7.88	1.63	1.77
10	1	96.9	92.7	9.53	95.3	0.383	24.9	1.40	6.81
30	3	99.6	61.2	11.1	36.8	0.417	26.6	1.05	10.6
100	10	83.1	55.6	28.9	28.9	0.517	55.9	1.22	23.7

[a] With purity as given in "purity fraction 1".

TABLE III

RESULTS OBTAINED WITH 5-μm STATIONARY PHASE AND MOBILE PHASE HEXANE–*tert.*-BUTYL METHYL ETHER (3:1)

Details as in Table II.

Mass injected per compound (mg)	Load per compound (mg g^{-1})	Purity fraction 1 (%)	Purity fraction 2 (%)	Amount fraction 1 (mg)	Yield compound 1 (%)	Width both fractions (min)	Throughput compound 1 (mg min^{-1})	Width fraction 1 (ml)	Concentration compound 1 (mg ml^{-1})
0.1	0.01	100	100	0.1	100	0.433	0.231	2.33	0.043
1	0.1	100	98.1	0.981	98.1	0.458	2.14	2.75	0.357
3	0.3	99.6	97.6	2.938	97.9	0.500	5.88	2.56	1.15
10	1	99.2	95.9	9.65	96.5	0.600	16.1	2.56	3.76
30	3	99.8	83.0	23.9	79.7	0.767	31.2	2.10	11.4
100	10	93.4	70.5	64.2	64.2	0.933	68.8	1.81	35.5

TABLE IV

RESULTS OBTAINED WITH 5-μm STATIONARY PHASE AND MOBILE PHASE HEXANE–*tert.*-BUTYL METHYL ETHER (9:1)

Details as in Table II.

Mass injected per compound (mg)	Load per compound (mg g^{-1})	Purity fraction 1 (%)	Purity fraction 2 (%)	Amount fraction 1 (mg)	Yield compound 1 (%)	Width both fractions (min)	Throughput compound 1 (mg min^{-1})	Width fraction 1 (ml)	Concentration compound 1 (mg ml^{-1})
0.1	0.01	100	100	0.1	100	0.700	0.143	4.67	0.021
1	0.1	100	98.4	0.984	98.4	0.867	1.14	4.67	0.211
3	0.3	99.5	98.0	2.95	98.5	1.03	2.86	4.90	0.602
10	1	100	90.1	8.90	89.0	1.38	6.45	4.67	1.91
30	3	97.6	78.6	22.5	75.1	1.77	12.7	4.44	5.07
100	10	79.0	69.5	80.4	80.4	2.43	33.1	5.70	14.1

TABLE V

RESULTS OBTAINED WITH 25-μm STATIONARY PHASE AND MOBILE PHASE HEXANE–*tert.*-BUTYL METHYL ETHER (1:1)

Details as in Table II.

Mass injected per compound (mg)	Load per compound (mg g^{-1})	Purity fraction 1 (%)	Purity fraction 2 (%)	Amount fraction 1 (mg)	Yield compound 1 (%)	Width both fractions (min)	Throughput compound 1 (mg min^{-1})	Width fraction 1 (ml)	Concentration compound 1 (mg ml^{-1})
0.1	0.01	100	84.5	0.0817	81.7	0.833	0.0981	1.98	0.041
1	0.1	99.0	80.9	0.773	77.3	0.967	0.800	2.10	0.368
3	0.3	98.7	78.5	2.21	73.8	0.983	2.25	1.75	1.26
10	1	99.1	71.0	5.99	59.9	1.07	5.60	1.52	3.94
30	3	99.5	62.2	11.9	39.5	1.13	10.5	1.28	9.30
100	10	90.7	55.7	24.6	24.6	1.17	21.0	1.40	17.6

TABLE VI

RESULTS OBTAINED WITH 25-μm STATIONARY PHASE AND MOBILE PHASE HEXANE–*tert.*-BUTYL METHYL ETHER (3:1)

Details as in Table II.

Mass injected per compound (mg)	Load per compound (mg g^{-1})	Purity fraction 1 (%)	Purity fraction 2 (%)	Amount fraction 1 (mg)	Yield compound 1 (%)	Width both fractions (min)	Throughput compound 1 (mg min^{-1})	Width fraction 1 (ml)	Concentration compound 1 (mg ml^{-1})
0.1	0.01	100	88.0	0.0864	86.4	1.43	0.0604	3.38	0.0256
1	0.1	100	85.4	0.829	82.9	1.37	0.605	3.03	0.274
3	0.3	99.4	82.4	2.38	79.2	1.57	1.51	3.03	0.785
10	1	99.3	79.1	7.42	74.1	1.73	4.29	2.68	2.77
30	3	98.4	76.1	21.0	70.1	1.90	11.1	1.98	10.6
100	10	96.4	61.1	38.6	38.6	2.20	17.5	1.86	20.8

TABLE VII

RESULTS OBTAINED WITH 25-μm STATIONARY PHASE AND MOBILE PHASE HEXANE–*tert.*-BUTYL METHYL ETHER (9:1)

Details as in Table II.

Mass injected per compound (mg)	Load per compound (mg g^{-1})	Purity fraction 1 (%)	Purity fraction 2 (%)	Amount fraction 1 (mg)	Yield compound 1 (%)	Width both fractions (min)	Throughput compound 1 (mg min^{-1})	Width fraction 1 (ml)	Concentration compound 1 (mg ml^{-1})
0.1	0.01	100	75.6	0.0677	67.7	1.83	0.037	5.13	0.013
1	0.1	100	82.6	0.789	78.9	2.20	0.359	4.89	0.161
3	0.3	99.4	82.1	2.36	78.8	2.53	0.933	4.66	0.506
10	1	99.3	80.8	7.69	76.9	2.78	2.78	4.31	1.78
30	3	99.1	76.0	20.8	69.2	3.50	5.94	3.61	5.76
100	10	98.1	62.2	40.5	40.5	4.40	9.20	2.80	14.5

phase. The fractions of less than 100% purity are contaminated by the other ketone, and because the two solutes are always injected as a 1:1 mixture the absolute amount of fraction 1 in milligrams is given by

$$A + b = M \cdot \frac{2P_2 - 1}{P_1 + P_2 - 1}$$

where A = mass of compound 1 in fraction 1; a = mass of compound 1 in fraction 2 (see below); B = mass of compound 2 in fraction 2; b = mass of compound 2 in fraction 1; M = injected mass of each compound, with $M_{\text{cyclohexanone}} = M_{\text{cyclopentanone}} = M$; and P = purity of fraction 1 or 2.

The expression for $A + b$ is found by a combination of the four equations $A + a = M$, $B + b = M$, $A/(A + b) = P_1$ and $B/(B + a) = P_2$. The total amount of fraction 2, $B + a$, could be calculated by an analogous equation. If the sample were not a 1:1 mixture of the two ketones, the equation for the calculation of $A + b$ would be much more complicated.

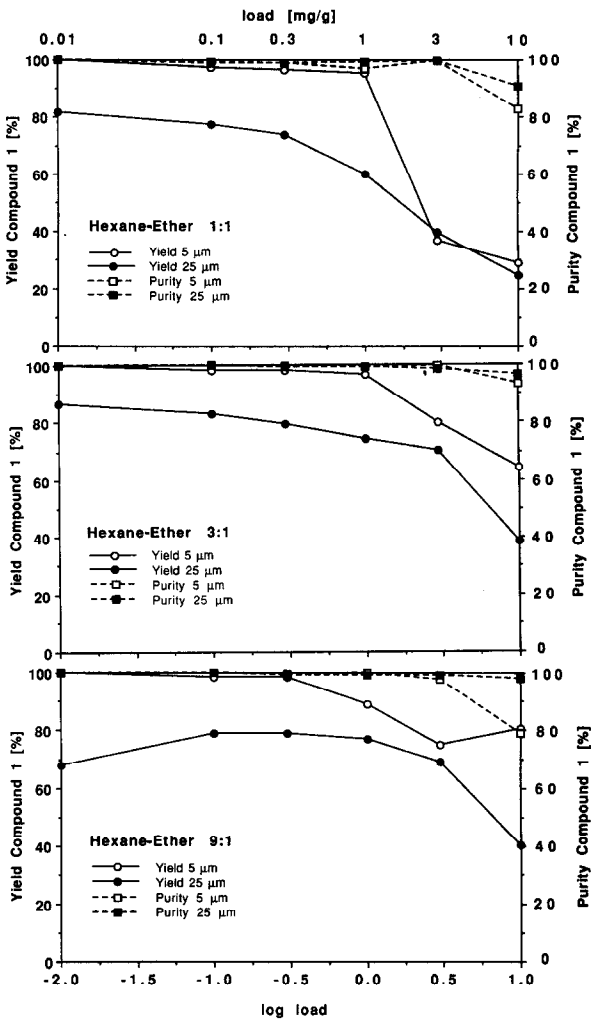

Fig. 2. Recovery yield and purity of compound 1 (cyclohexanone) *vs.* increasing load on 5- and 25-µm silica with hexane–*tert.*-butyl methyl ether mixtures of various compositions as mobile phase.

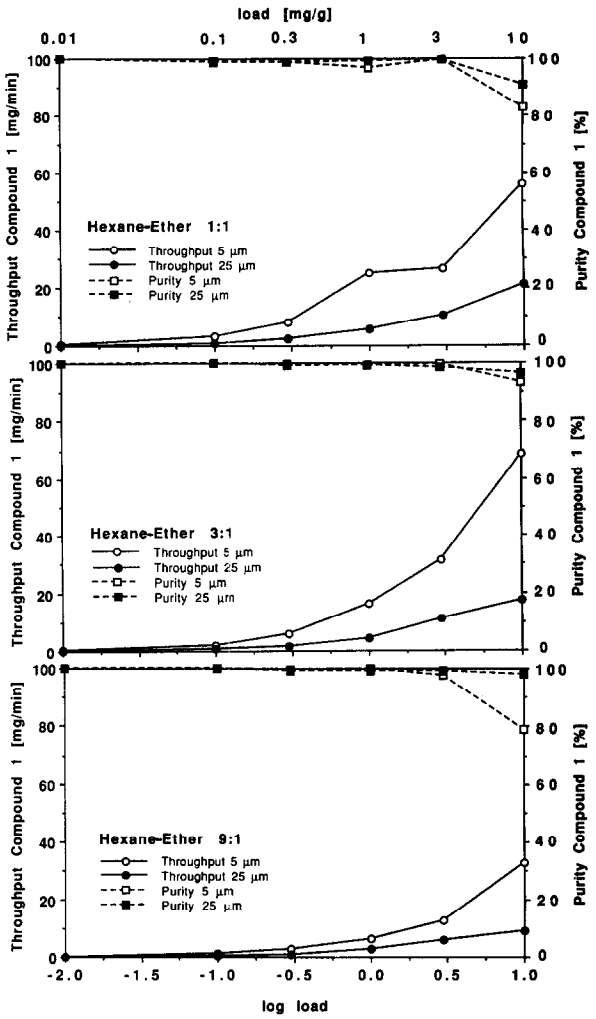

Fig. 3. Throughput and purity of compound 1 (cyclohexanone) *vs.* increasing load on 5- and 25-µm silica with hexane–*tert.* butyl methyl ether mixtures of various compositions as mobile phase.

The calculation of the recovery yield of compound 1 (with purity as given in the third column of the tables) is obvious. Its throughput is given by dividing the amount of fraction 1 by the width of both fractions in minutes. This approach presupposes that consecutive injections are nested in order to give touching peak pairs, *i.e.*, peaks other than those for the two ketones are absent. For a correct calculation of throughput, the retention time of the first injection should also be considered, which is not the case in the tables.

In the last column of the tables the concentration of compound 1 is listed, which can easily be calculated if the width (volume) of fraction 1 is known.

Fig. 2 summarizes the recovery yield and purity of compound 1 and Fig. 3 shows the throughput and purity.

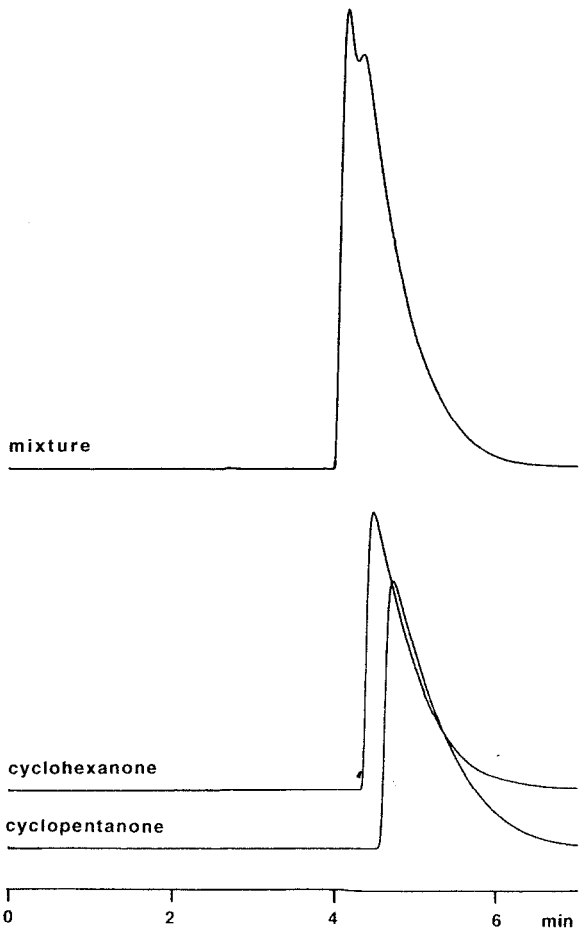

Fig. 4. Elution profiles of the individual compounds and of a 1:1 mixture of the two ketones. Sample, 100 mg of each component, *i.e.*, 200 mg of mixture, dissolved in the same amount of mobile phase; stationary phase, 25-μm silica; mobile phase, hexane–*tert.* butyl methyl ether (3:1); flow-rate, 7 ml min^{-1}.

DISCUSSION

The displacement effect manifests itself by the fact that the width of fraction 1 decreases with increasing load (with the exception of the 5-μm silica and 9:1 mobile phase composition). Without a displacement effect, the volume of this fraction would be constant and any shifting of retention times of the two ketones would be expected to be parallel. Fig. 4 shows that cyclohexanone is eluted earlier and as a narrower peak (of 96% purity) when injected as a mixture in comparison with the peak profiles of the individual compounds. A similar result was found by Newburger and Guiochon [5] for the diethyl phthalate–β-tetralone pair. For effective self-displacement, they stated that the amount of the second-eluted compound should be higher than of the first-eluted compound.

With increasing load, the purity of both fractions decreases, *i.e.*, a certain amount of compound is "lost" as an impurity in the fraction of the other ketone. Therefore, the recovery yield of compound 1 decreases. Note that its yield can be low, although its purity is high. The purity of fraction 1 is always markedly higher than that of fraction 2. As already mentioned, this approach is not economical if the second-eluted compound, cyclopentanone, needs to be obtained in pure form.

Regarding the throughput of compound 1, this increases with increasing load at the expense of purity and recovery yield. The concentration of compound 1 also increases, which is desirable because the isolation of the product by evaporation of the mobile phase is easier and possible contamination from eluent impurities is lower.

Influence of mobile phase composition

As can be seen from Table I, decreasing the ether content and therefore the polarity of the mobile phase entails higher capacity factors, k', and, as expected, better resolution, R, of the peaks. However, this latter effect is less pronounced, as would be expected owing to the observed decrease in the

separation factor, α. Also, the tailing, T, increases with decreasing mobile phase polarity, a feature typically found with normal-phase separations on silica. For preparative separations it is highly desirable to find conditions where the separation factor and resolution (under analytical conditions) are high whereas tailing would be low. This is not possible here, and therefore Fig. 2 shows no clear trend that the purity and yield of compound 1 increase with decreasing ether content. It seems as if the mobile phase of medium polarity would be the best compromise. Concerning throughput, the results are clearer. Owing to increased peak widths with lower polarity, the throughput decreases. Any effect of mobile phase strength on capacity factors (retention times) has no influence on throughput if this is calculated by the method given above, *i.e.*, with consecutive and nested injections. Concerning the sample concentration in the eluate, the mobile phase of medium strength is best for both types of stationary phase.

Influence of stationary phase particle size

The 25-μm silica gives a higher purity of fraction 1 whereas with the 5-μm phase the purity of fraction 2 is higher (or its impurity is lower). This is the reason why the yield of compound 1 is higher with the fine silica (but with lower purity). Also, the throughput of compound 1 is higher on the 5-μm phase (again with lower purity). The throughput on the coarse stationary phase is only *ca.* 30% of that on the fine material. If the 25-μm column were also used at a flow-rate of 14 ml min^{-1}, its throughput could be expected to be *ca.* 60% but perhaps with lower purity, considering the earlier studies on van Deemter plots at mass overload [7]. In contrast, Guiochon and co-workers found that the conditions for maximum productivity are far from the van Deemter optimum [5,10].

CONCLUSIONS

This study provides further confirmation that of purity, yield and throughput, only two parameters can be kept high at the expense of the third. The calculation of the amount of fraction mass presented here, which is possible from the amount injected and the fraction purities, is the key for the determination of yield, throughput and product concentration. Concerning the size of the stationary phase, no general recommendations can be given but as the mobile phase hexane and *tert.*-butyl methyl ether (3:1) is often the best compromise.

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