Concentration of small volumes of nonpolar solutions containing trace volatile compounds

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Abstract

The analytical behaviour of the concentration of extracts under a stream of nitrogen of small volumes of hexane solutions containing volatile analytes has been studied. The proportion of analyte blown off the solution is a function of its boiling point, of its polarity, and of the final volume reached. For similar boiling points, the more polar the compound, the higher the level of losses. If the evaporation progresses to a final volume of 0.01 ml, all the compounds with boiling points below 150°C are lost in proportions higher than 50%. Compounds with boiling points between 150 and 250°C are lost in proportions ranging from 70 to 25%, and only eicosane (b.p. 340°C) is almost fully retained in the solution. The addition of small amounts (5 mg) of dehydrated silica to the evaporating solution allows the evaporation to proceed without noticeable losses of solutes more polar than hydrocarbons. After concentration, the solutes can be easily recovered by adding a small amount of ethanol or a mixture water–ether. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

There are several steps commonly included in trace volatile analytical procedures that have received rather less attention than should have been expected. One of these steps is the concentration of extracts through solvent evaporation. In a previous paper [1], the analytical characteristics of a micro-Kuderna-Danish concentrator were described and the conclusions reached showed that the losses become critical in the last steps of the concentration of dilute solutions. It became clear, however, that if properly carried out, concentration of 20–30 ml solutions up to a final volume of 0.5 ml can be done without altering the analytical characteristics of the sample

significantly. Quite a different problem is the one encountered when 1 or 2 ml of solution must be concentrated to a final volume of 0.1 ml or to an even smaller one. In this case, the situation changes completely due to several reasons. Firstly, there is no standard equipment specifically designed to handle these volumes. Secondly, the mass transfer characteristics of small volumes are not the same as those of larger ones. Thirdly, and as a consequence of these two previous reasons, it is very difficult to perform fractional distillation. Hence, the concentration of small volumes is usually carried out by evaporating the solvent under a stream of purified Nitrogen [2], or by simple microdistillation [3,4].

In a classic work, Grob and Müller [5] compared the performance of several strategies for concentrating small volumes (from 800 to 10 μl) of pentane

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solutions. Their results showed that solvent evaporation in a stream of nitrogen only caused small amounts of solute material to co-evaporate (8–15%), and that the losses were independent of the boiling points of the components. Still better results were obtained via microdistillation of the solutions. These results may lead us to think that the concentration of a small volume of a nonpolar solution would not pose any problem. However, the conditions in which these essays were done cannot often be met in practice. First of all, the solvent used is pentane, which has a rather low boiling point (36°C), while it is very often necessary to use hexane (b.p. 68°C) or even isooctane (b.p. 118°C) as solvents, particularly if a HPLC operation is involved. Secondly, the test analytes considered in that study were very nonpolar compounds, mainly hydrocarbons or long-hydrocarbon chain esters, whose pentane or hexane solutions behave quite ideally, behaviour that cannot be expected in solutions containing compounds with a wider range of polarities.

In fact, our experience has shown us [6] that the concentration of small volumes of hexane solutions is very problematic, and that a significant proportion of volatile material can be lost. This is a rather large limitation since hydrocarbons or mixtures of active-solvent–hydrocarbon are becoming increasingly popular for environmental and safety reasons and because they are a common choice as components of the mobile phases used in normal-phase prefractionation of flavours or other volatile extracts [6–8]. In this paper we have studied the analytical behaviour of these concentration systems, the effect that several factors exert on the losses of volatile compounds, and the possibilities of controlling the loss of volatile compounds through the addition of small amounts of adsorbents to the solution.

2. Experimental

2.1. Reagents

All solvents were supplied by Scharlau (Barcelona, Spain) and were Analytical Grade. Silica gel 60 from Merck (Darmstadt, Germany) was reactivated by heating at 160°C for 24 h. This was named silica 0. Silica activity 10, 20, 30 and 35 were prepared by adding 10, 20, 30 or 35% of water (v/w), respectively, over silica 0. All the pure compounds used in this study were purchased from Polyscience Corporation (Niles, IL, USA), and when available, were of analytical grade.

Internal standard solution: 0.05 g of 3-methyl-2-pentanol and 2-octanol in 25 ml of hexane.

2.2. Gas chromatography

HP 5890 series II gas chromatograph, fitted with Split–Splitless injector, and an automatic sampler HP 7673 A.

Column: Supelcowax 10: 60 m×0.32 mm I.D., and 0.5 mm film thickness.

Chromatographic conditions: Splitless injection, Carrier H_2, head pressure 120 KPa, Split flow 27 ml min^{-1}, Purge flow 3 ml min^{-1}, Splitless time 1.5 min. Injected volume 1 μl. Split Injection, As above except Split flow was 65 ml min^{-1}.

Injector and detector temperatures were 250°C. Initial column temperature 40°C held for 10 min, and then raised to 190°C at 4°C min^{-1}. Make up gas N_2 at 30 ml min^{-1}. Detector FID

2.3. Basic procedure for the concentration of hexane solutions

Samples of 2 ml were placed in a 10-ml test tube with a conical bottom. The tubes were partially submerged in a water bath at 22.5°C and a thin stream of 90±5 ml/min of nitrogen was then directed to the center of the surface of the solution. The evaporation was allowed to continue until the desired final volume was achieved. Then the tube was removed from the system, 5 μl of the Internal Standard Solution were added, the mixture transferred to a vial, its volume adjusted to 200 μl and analysed.

2.4. Adsorption of solutes by silica

Sets of duplicate tests tubes containing different amounts (2, 5, 10 and 15 mg) of silica of different activities (0, 10, 20, 30 and 35) and 100 μl of a hexane solution containing 6 mg/l of the analytes

were prepared. Each tube was carefully shaken for 15 min avoiding any splashing of the solution on the tube walls, and was later centrifuged at 1000 rpm for 5 min to settle the silica in the bottom. 60 μl of the supernatant were transferred to a vial, 5 μl of the Internal Standard solution were added and then analysed.

2.5. Desorption of solutes

Sets of duplicate tests tubes containing 5 mg of dehydrated silica and 20 μl of a hexane solution containing 30 mg/l of the analytes were prepared and shaken softly for 15 min to allow equilibriation. Over that volume, 80 μl of different solvents (hexane, dichloromethane, ether, and ethanol) were added plus 5 μl of water (except in the case of ethanol). After re-equilibration, the tubes were centrifuged and analysed.

2.6. Recommended procedure

Weigh 5 mg of previously dried silicagel in a conical tube and pour in the solution to be concentrated (typically 2 ml). Shake the tube softly and after a 10 min equilibration time, centrifuge it at 1000 rpm for 1 min to settle the silica in the bottom. Concentrate the solution under a high purity N_2 stream (90±5 ml/min) until near dryness (about 10 microlitres), taking care that the solution does not become dry. Then add 50 microlitres of pure ethanol to desorb the volatile compounds (alternatively 50 μl of ether plus 5 μl water), shake softly and centrifuge again (1000 rpm×2 min) to recover the ethanol by transferring it to a vial.

Important note: The chromatographic behaviour of ethanol is problematic if columns with nonpolar or slightly polar stationary phases are used together with conditions leading to solvent recondensation (initial column temperatures below 76°C). If this is
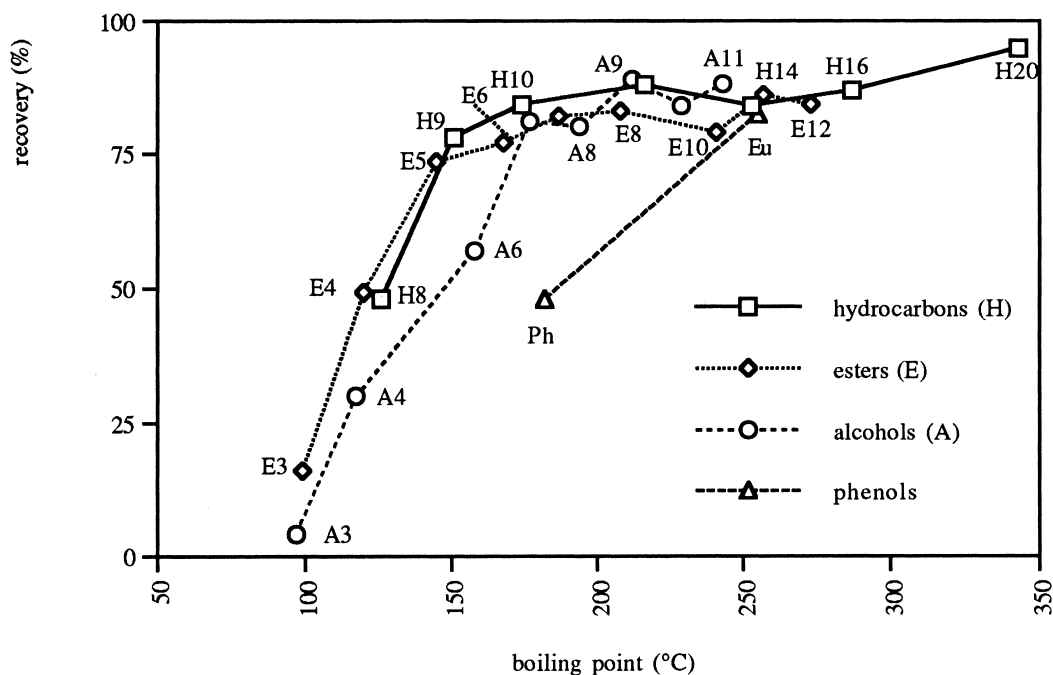

Fig. 1. Percent of material retained in the hexane solution vs. analyte boiling point, after concentration of a 2 ml volume to a final volume of 0.1 ml. Compound codes: H8=*n*-octane; H9=*n*-nonane; H10=*n*-decane; H12=*n*-dodecane; H14=*n*-tetradecane; H16=*n*-hexadecane; H20=*n*-eicosane; E3=ethyl propanoate; E4=ethyl butyrate; E5=ethyl pentanoate; E6=ethyl hexanoate; E7=ethyl heptanoate; E8=ethyl octanoate; E10=ethyl decanoate; E11=ethyl undecanoate; E12=ethyl dodecanoate; A3=*n*-propanol; A4=*n*-butanol; A6=*n*-hexanol; A7=*n*-heptanol; A8=*n*-octanol; A9=*n*-nonanol; A10=*n*-decanol; A11=*n*-undecanol; Ph=phenol; Eu=Eugenol.

the case, the problems can be overcome by the use of a 2 m retention gap deactivated with phenylsiloxane.

3. Results and discussion

3.1. Concentration of nonpolar solutions

In a preliminary part of the work, different conditions for the concentration system were tested to determine those leading to the lowest losses of analytes in the concentration process. In order to do that, a series of experiments comprising different system geometries, water bath temperatures and flows of nitrogen, were carried out. The results demonstrated that if those variables have any influence, there is a minimum level of losses that cannot be reduced just by further optimization of these variables. In fact, the flow of nitrogen is important only if it is large enough to provoke

projections of liquid material onto the dry walls of the concentration system (which happens at flows higher than 120 ml/min), and the temperature increases the amount of material blown off the solution only if it is higher than 40°C. Below these 'critical' values of nitrogen flow and water bath temperature, these variables do not exert any influence on the level of analytes lost in the process but on its speed; and the lower they are, the higher the time needed to concentrate the solutions. Therefore, the conditions of nitrogen flow and temperature used in the system described in the experimental section, represent a reasonable optimum which, however, involves the loss of volatile material.

With regard to the geometry and design of the system, the results obtained by using different microdistillation techniques did not outperform the ones obtained by using the simplest evaporation under nitrogen. Furthermore, if room temperatures are to be used to prevent artifact formation, the use of mi-
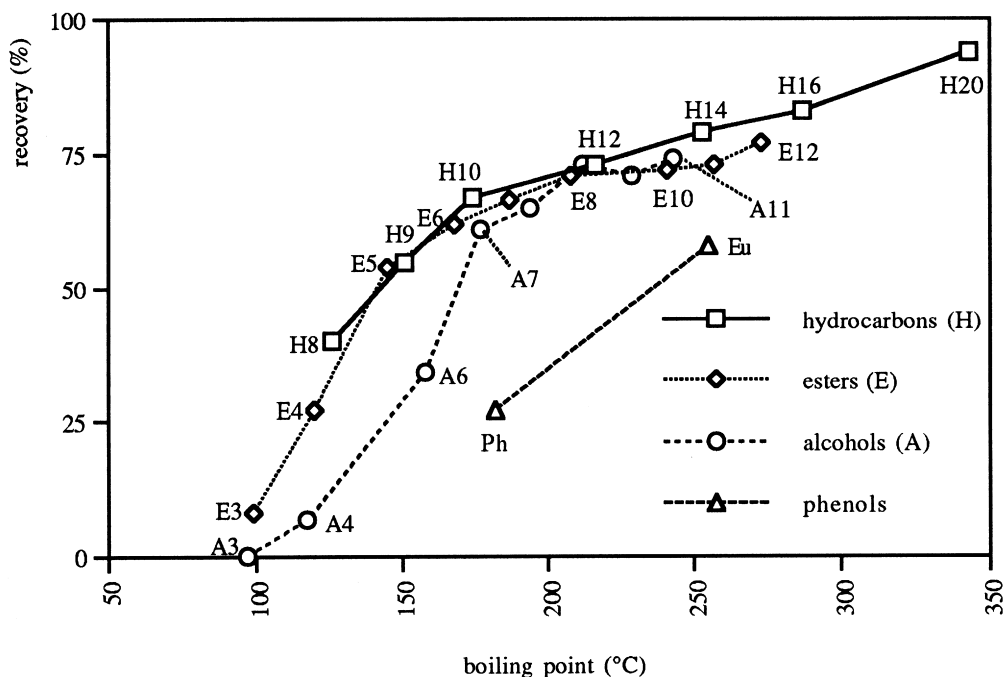

Fig. 2. Percent of material retained in the hexane solution vs. analyte boiling point, following the concentration of a 2 ml volume to a final volume of 0.01 ml. Compound codes: H8=*n*-octane; H9=*n*-nonane; H10=*n*-decane; H12=*n*-dodecane; H14=*n*-tetradecane; H16=*n*-hexadecane; H20=*n*-eicosane; E3=ethyl propanoate; E4=ethyl butyrate; E5=ethyl pentanoate; E6=ethyl hexanoate; E7=ethyl heptanoate; E8=ethyl octanoate; E10=ethyl decanoate; E11=ethyl undecanoate; E12=ethyl dodecanoate; A3=*n*-propanol; A4=*n*-butanol; A6=*n*-hexanol; A7=*n*-heptanol; A8=*n*-octanol; A9=*n*-nonanol; A10=*n*-decanol; A11=*n*-undecanol; Ph=phenol; Eu=Eugenol.

crodistillation apparatus becomes complicated and highly impractical.

The amount of material lost in the concentration process depends on three factors: the boiling point of the compound, its polarity, and the final volume reached in the concentration process, as expressed in Figs. 1 and 2. These figures represent the proportion of material retained in the solution following concentration to final volumes of 0.1 and 0.01 ml respectively versus the boiling point of the compounds. For the sake of clarity, the compounds have been classified into chemical classes. The magnitude of the losses of solutes in these concentration processes is worth mentioning since they can be higher than 50%, even in the case of some compounds with boiling points higher than 150°C, that is, 90°C above the solvent boiling point. Furthermore, compounds with boiling points well above 200°C are still lost in proportions near 25%, as can be seen in Fig. 2 and, in fact, the only compound of the experiment which was almost fully retained in the evaporating solution was eicosane (whose boiling point is 340°C).

The relationship between boiling points and proportion of material recovered depends on the polarity of the chemical, although in all the chemical classes a similar trend is observed. In the lower range of boiling points, a small difference in the boiling points accounts for a large difference in the recoveries, while above a certain point this dependence softens. These inflection points range from 160 to 200°C, depending on the chemical series. Anyway, an inspection of Figs. 1 and 2 allows us to state that the smaller the final volume, the higher the range of influence of the boiling points on the proportion of material recovered. The role played by polarity is clearer in the lower range of boiling points, and manifests itself in the fact that the more polar the compound, the higher the losses. This can be clearly seen when comparing the cases of nonane (B.P.= 151°C; R=77%), n-hexanol (B.P.=158°C; R=58%) and phenol (B.P.=182°C; R=43%).

Finally, the reproducibility of the process depends on the final volume reached, but not on the nature of analytes, as can be seen in Table 1. Reproducibility, expressed as % of material recovered, ranges from 5 to 50, 12 being the average value when the concentration process progresses until 0.01 ml. Data in

Table 1
Influence of the final volume reached on the reproducibility of the process

Compound	Reproducibility[a]	
	Final volume 100 µl	Final volume 10 µl[b]
n-Octane	4.91	19.2[d]
n-Nonane	5.77	17.2[d]
Ethyl propanoate	6.07	10.4
n-Decane	8.57	50.1[d]
Ethyl butyrate	6.95	17.4[d]
n-Propanol	4.80	4.72
Ethyl pentanoate	7.97	16.9[c]
n-Butanol	7.23	16.3[c]
n-Dodecane	11.4	13.8
Ethyl hexanoate	5.30	10.9[c]
Ethyl heptanoate	7.30	9.17
n-Hexanol	17.4	16.9
n-Tetradecane	9.76	13.0
Ethyl octanoate	13.9	22.7
n-Heptanol	10.8	6.31
n-Octanol	8.62	15.1
n-Hexadecane	12.9	23.5
Ethyl decanoate	7.38	15.3[c]
n-Nonanol	6.81	14.6[c]
Ethyl undecanoate	6.88	11.7
n-Decanol	11.4	9.68
Ethyl dodecanoate	8.43	17.9[c]
n-Undecanol	9.24	11.6
n-Eicosane	6.71	14.4[c]
Phenol	8.93	16.1
Eugenol	6.40	12.6[c]

[a] Reproducibility was obtained in an Analysis of Variance (ANOVA) experiment in which four sets of four solutions each were concentrated on different working days. It has been estimated as the standard deviation of the four daily average recoveries, and multiplied by the square root of 4.
[b] A Fischer F test was carried out to check whether there are differences in repoducibility linked to the final volume:
[c] Significant at $P > 0.9$.
[d] Significant at $P > 0.95$.

the table suggest that the relative imprecision of the process will be directly proportional to the amount of material lost, and that it will be too high for those analytes which are less retained in the process.

3.2. Discussion

Some of the aforementioned observations do not fit a model which only considers ideal vapour–liquid equilibria. In particular, such a model cannot explain:

(1) why chemical compounds with different functionalities but with similar boiling points and vapour pressures are retained to different extents, nor (2) why components with very high boiling points or very low vapour pressures are lost to such an appreciable extent.

The first question can be answered by taking into account that, in the case of a polar compound, neither the vapor pressure of the pure compound nor its boiling point actually represent the volatility of that compound in a nonpolar solution, since in this kind of solution polar interactions can hardly take place. Consequently, the volatility of polar compounds is underestimated if measured through their boiling points or the vapour pressures of the pure compounds. This could be clearly demonstrated by replacing the boiling point by the Kovats Index of the compound on a nonpolar column in Fig. 1 or Fig. 2. This effect is shown in Fig. 3, which reveals that the differences between chemical functionalities have almost disappeared.

In order to explain the second question, it is necessary to use the concept of coevaporation [1,5]. This concept states that if the analytes are deposited on a clean, dry glass surface with quite a low retention power, they will be easily carried out of the evaporating solution by the stream of ascending solvent, almost regardless of their boiling point. According to this concept, the least volatile material is lost due to coevaporation, while the most volatile compounds are lost due to both: coevaporation and vapour partition.

3.3. The use of silica to reduce the losses during the concentration process

An effective way to reduce the losses of analytes during the concentration process is to reduce the proportion of compound that is free in the solution, which can in turn be achieved by adding a small amount of adsorbent to the solution. We have studied the effect of the addition of different amounts of
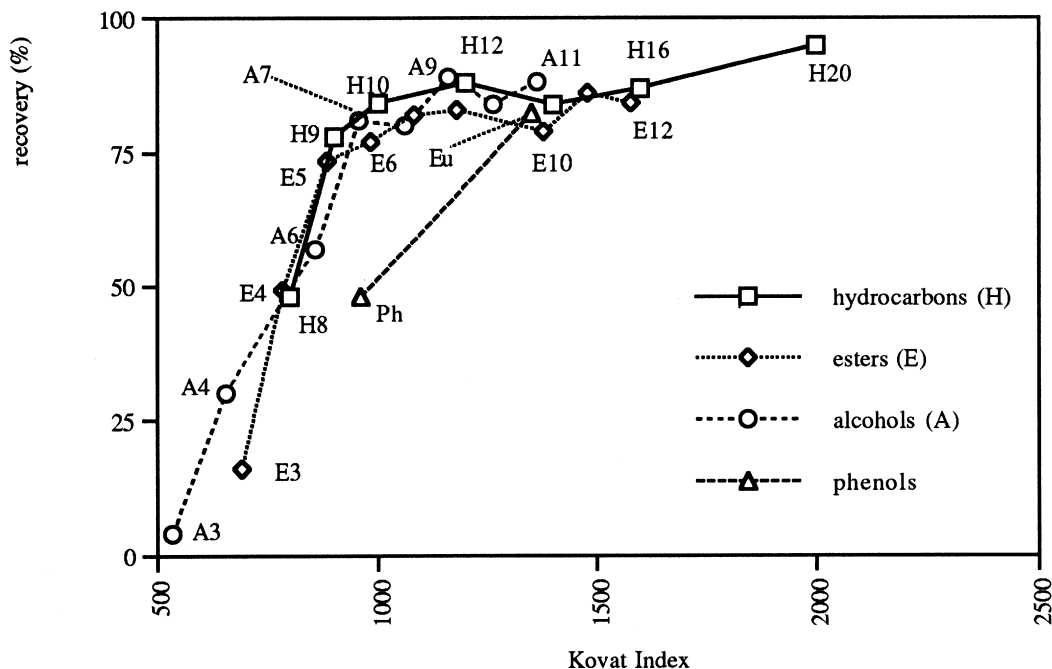

Fig. 3. Percent of material retained in the hexane solution, vs. the Kovats Index in nonpolar columns, following the concentration of a 2 ml volume to final volume of 0.1 ml. Compound codes: H8=*n*-octane; H9=*n*-nonane; H10=*n*-decane; H12=*n*-dodecane; H14=*n*-tetradecane; H16=*n*-hexadecane; H20=*n*-eicosane; E3=ethyl propanoate; E4=ethyl butyrate; E5=ethyl pentanoate; E6=ethyl hexanoate; E7=ethyl heptanoate; E8=ethyl octanoate; E10=ethyl decanoate; E11=ethyl undecanoate; E12=ethyl dodecanoate; A3=*n*-propanol; A4=*n*-butanol; A6=*n*-hexanol; A7=*n*-heptanol; A8=*n*-octanol; A9=*n*-nonanol; A10=*n*-decanol; A11=*n*-undecanol; Ph=phenol; Eu=Eugenol.

silica of different activities to the evaporating solution. The amount of silica that can be added is limited by practical reasons, and if the final volume must be below 100 μl, no more than 5 mg of silica should be added to the tube. Under these conditions, the best results are achieved with fully dehydrated silica. The proportions of solutes which are not adsorbed on the silica surface are given in Table 2. As can be seen in the table, the addition of silica does not have, as expected, any effect on the hydrocarbons, but has a very noticeable effect on the other compounds. For instance, more than 85% of all the ethyl esters, which are only slightly polar compounds, are adsorbed on the silica surface. The case of alcohols and phenols is clearer, and more than

Table 2
Liquid solid equilibria: Percent of material remaining free in a 100 μl hexane solution containing 5 mg of fully activated silica

Compound	Average ($n=16$)	Reproducibility[a]
n-Octane	100	2.29
n-Nonane	100	2.18
Ethyl propanoate	14.9	2.26
n-Decane	101.6	3.67
Ethyl butyrate	13.3	1.66
n-Propanol	1.50	0.87
Ethyl pentanoate	15.2	1.84
n-Butanol	1.28	0.38
n-Dodecane	99.9	0.10
Ethyl hexanoate	12.4	1.85
Ethyl heptanoate	11.9	1.75
n-Hexanol	2.42	0.70
n-Tetradecane	100	1.63
Ethyl octanoate	11.9	2.36
n-Heptanol	2.30	0.46
n-Octanol	1.96	0.63
n-Hexadecane	101	2.79
Ethyl decanoate	13.1	1.49
n-Nonanol	2.63	1.63
Ethyl undecanoate	14.5	3.56
n-Decanol	2.30	1.05
Ethyl dodecanoate	13.5	1.14
n-Undecanol	2.08	1.07
n-Eicosane	100	3.29
Phenol	3.87	1.32
Eugenol	5.08	1.61

[a] Reproducibility was obtained in an Analysis of Variance (ANOVA) experiment in which four sets of four solutions each were analyzed on different working days. It has been estimated as the standard deviation of the four daily average results, and multiplied by the square root of 4.

95% of all these compounds are retained on the silica surface. All this means that, with the exception of hydrocarbons, virtually all the material present in the evaporating solution will be absent from the evaporating liquid.

The next problem to tackle is how to recover the analytes that are now retained on the silica surface. The only way to do it is by means of competitive adsorption of a third compound. Results for three different compounds and four different strategies are summarised in Fig. 4. The figure represents the percent of solute that remains free in a 100 μl solution containing 5 mg of silica. It can be seen that the addition of a small amount of water (5 μl) directly to the hexane layer containing the silica releases more than 50% of the adsorbed material. If the hexane is replaced by dichloromethane (plus 5 μl of water), the esters are fully released from their association with silica, while recovery of alcohols and phenols is better than 80%. Still better results are obtained when ether is used (plus 5 μl of water), which guarantees more than 95% of recovery for all the solutes tested, but the best results were achieved with ethanol, which made an almost total recovery for all the compounds possible.

Finally, these studies allow us to define an optimum operative procedure for the concentration of small volumes of hexane solutions which is described in the experimental section (Section 2.6). The results obtained in the concentration of a 2 ml hexane solution to a final volume of about 50 μl (in ethanol) by following that procedure are given in Fig. 5. As can be seen in the figure, recoveries are now almost total for all the chemicals tested with the exception of hydrocarbons, which were not retained by the silica and followed a pattern very close to that observed in the direct concentration of hexane solutions. The corresponding reproducibility values are far better than those observed in the direct concentration of hexane solutions, and they were well below 6% for all the tested compounds with a fairly good long-term performance.

In conclusion, it can be said that the proposed strategy makes it possible to concentrate small volumes of nonpolar solutions by avoiding the losses and the irreproducibility normally linked to these processes. In addition, this strategy allows us to quantitatively transfer the solutes contained in a
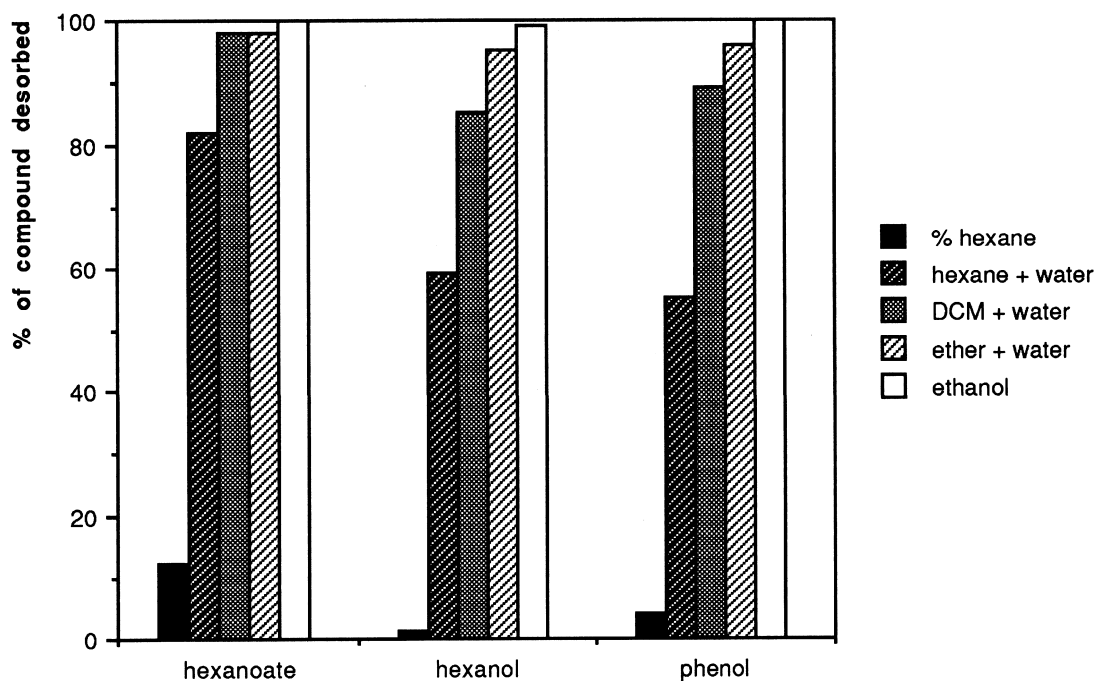
Fig. 4. Proportion of solutes desorbed by the addition of different solvents.
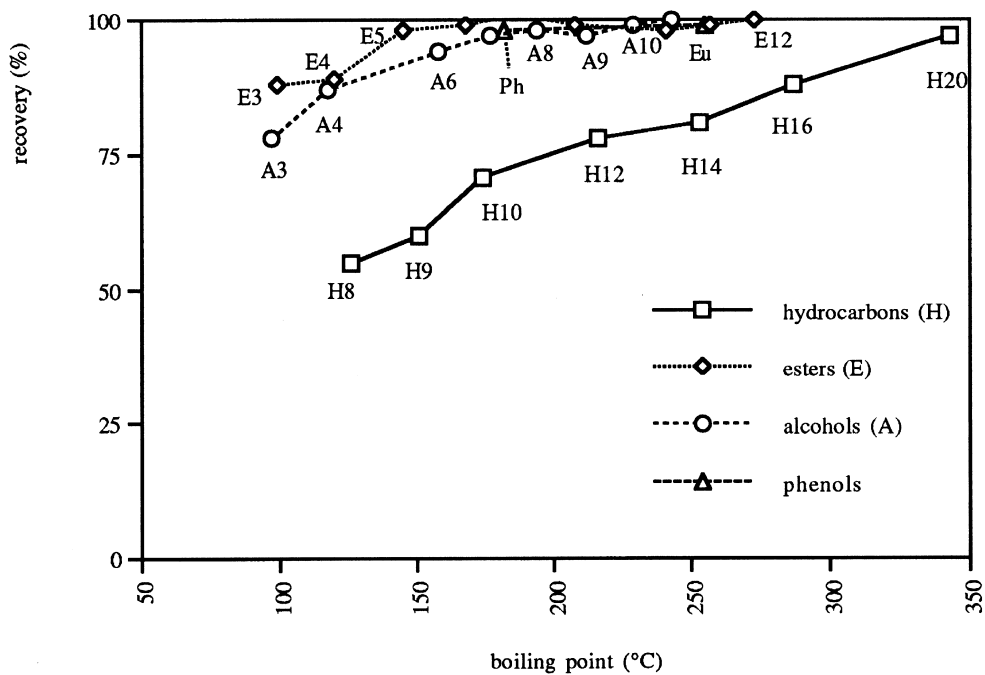

Fig. 5. Percent of material retained in the concentration of a 2 ml hexane solution following the proposed procedure. Compound codes: H8=n-octane; H9=n-nonane; H10=n-decane; H12=n-dodecane; H14=n-tetradecane; H16=n-hexadecane; H20=n-eicosane; E3=ethyl propanoate; E4=ethyl butyrate; E5=ethyl pentanoate; E6=ethyl hexanoate; E7=ethyl heptanoate; E8=ethyl octanoate; E10=ethyl decanoate; E11=ethyl undecanoate; E12=ethyl dodecanoate; A3=n-propanol; A4=n-butanol; A6=n-hexanol; A7=n-heptanol; A8=n-octanol; A9=n-nonanol; A10=n-decanol; A11=n-undecanol; Ph=phenol; Eu=Eugenol.

nonpolar solution to a polar one which can be used in subsequent analytical or sensory studies.

Acknowledgements

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