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ADSORPTION PROPERTIES OF STAINLESS-STEEL CAPILLARIES USED IN THE PREPARATION OF OPEN TUBULAR GAS CHROMATOGRAPHIC COLUMNS

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SUMMARY

Adsorption on the walls of stainless-steel capillary tubing has been studied by means of elution gas chromatography with substances of differing chemical functional groups. Several procedures for cleaning the column walls have been tested and their effect on the adsorption activity of the surface is described. By means of controlled cleaning, it is possible to prepare low-adsorptivity surfaces, the properties of which can be defined quite well in terms of capacity ratios and peak shapes. Satisfactory and reproducible column performance has been obtained with surfaces of this type coated with squalane as the stationary phase.

INTRODUCTION

The inner surface of capillary tubing used in the preparation of wall-coated open tubular gas chromatographic (GC) columns inevitably possesses a certain adsorptivity that can determine the overall chromatographic performance. Hence, tubes differing in their surface properties may yield columns having divergent behaviour. Such differences in behaviour can arise not only with different tubing materials (*e.g.*, glass, steel or nylon¹), but also with different samples of the same material. For example, changes in the relative retentions of aromatic hydrocarbons with respect to alkanes were observed on stainless-steel capillaries coated with the same stationary phase², and were attributed to different methods of manufacture of the tubing. Similar observations on varying retentions and varying extents of peak asymmetry for more polar compounds have been made on several occasions in our laboratory, even with steel tubes of identical origin; as all of the other parameters seemed to be constant, the probable cause was the surface activities of the walls of the tubes, a factor which normally escapes attention.

In an attempt to gain a better understanding of these phenomena, a study was undertaken of the adsorption interactions in "empty" (*i.e.*, uncoated) stainless-steel tubes on the basis of elution GC of substances having differing chemical functions. With metal tubing, however, there is (contrary to, for example, glass) a fundamental

difficulty in that the inside walls are contaminated primarily with drawing compounds used in the manufacturing process³. For this reason, the walls must be cleaned prior to coating.

Much attention has been given to the cleaning of metal capillaries since they were introduced into chromatographic work, but, nevertheless, there are no criteria which would enable the "cleanliness" of a surface to be judged. *A priori*, it may be reasoned that the milder procedures, involving organic solvents (and used by the majority of workers⁴⁻¹¹), need not always be able to remove all of the contaminating material, whereas the more drastic reagents, such as inorganic acids, ammonia or potassium hydroxide (used, eventually, in various combinations¹²⁻¹⁷), can result not only in "cleaning" but also in chemical changes in the surface layer of the metal. (It should be emphasized, however, that chemical modifications might also have a favourable effect on the wettability of the walls.)

The progress of cleaning is, if at all, usually controlled only by following the contamination of the liquid emerging from the tube, and the success of the treatment is judged after the coated column is finished. Only one study⁹ mentions an intermediate chromatographic test in which samples of test solutes were injected into the (uncoated) tube; a definite relation was established between the intensity of cleaning (using organic solvents) and the capacity ratios and peak shapes of *n*-hexane and benzene.

Similar problems also arise in the regeneration of old, used, open tubular columns as in the cleaning of new tubes. As the stationary phase may undergo chemical changes with time, one cannot be sure that the coating will be completely removed by simply rinsing the tube with a good solvent for the original substrate and that the surface will resume its previous character. Evidence has been found¹³ that a residual deposit of the original liquid is able to affect the performance of the re-coated column.

For these reasons, experiments with different cleaning methods had to be included in the present study. Finally, in order to confirm the relation between the activity of the tube walls and the behaviour of coated open tubular columns, selected capillaries were coated with squalane (as a well defined and generally used apolar phase) and their retention parameters were compared.

EXPERIMENTAL

Stainless-steel tubing from three different sources was investigated: (1) from Superior Tube, Norristown, Pa., U.S.A. with the manufacturer's designation S_m/S 30; (2) from Laboratorní Přístroje, Prague, Czechoslovakia; (3) from Carlo Erba, Milan, Italy. The internal diameter of the tubes was 0.25–0.26 mm and the length was 50 ± 2 m. Flushing of the capillaries (as well as coating with the stationary phase) was performed with the aid of nitrogen pressure in a special filling device, in which the liquids were allowed to come in contact only with glass. The basic portion of a washing liquid was 3 ml and the pressure was adjusted so as to force it through the tube in *ca.* 1.5 h.

The following cleaning methods were tested: (a) organic solvents in the sequence *n*-pentane, benzene, chloroform, acetone, chloroform, benzene and *n*-pentane; (b) nitric acid and ammonia according to Mon *et al.*¹², *i.e.*, in the sequence chloroform.

acetone, distilled water, concentrated nitric acid, water, concentrated ammonia, water, acetone, chloroform and *n*-pentane; (c) as in the preceding case, but omitting ammonia; (d) as in (b) but omitting nitric acid; (e) an aqueous solution of Komplexon III (10% w/w), followed by repeated rinsing with water and acetone (Komplexon III, the disodium salt of ethylenediaminetetra-acetic acid was tried as a strong complexing reagent for iron).

Squalane used for the coated columns was from B.D.H., Poole, Great Britain or from Schuchardt, Munich, G.F.R.; no difference was observed between the two samples. The columns were prepared by the standard dynamic method using solutions in *n*-pentane. After coating, the columns were heated slowly up to 60° under a nitrogen flow until the background current of the flame ionization detector remained constant; they were then considered to be ready for measurements.

A Chrom 4 gas chromatograph (Laboratorní Přístroje, Prague) with a flame ionization detector was used; nitrogen was the carrier gas. The adsorption tests were carried out at a constant oven temperature of 60° (injection chamber held at 180°), and at a constant mean gas velocity of 10.5 ± 0.5 cm/sec. Special attention was paid to the cleanliness of the injection port and the splitting system. The sorbates were injected at three concentration levels, the amount of a single compound injected being 0.02, 0.05 and 0.50 μ l; using the splitting ratio 1:1000, this corresponds to amounts of ca. $2 \cdot 10^{-8}$, $5 \cdot 10^{-8}$ and $5 \cdot 10^{-7}$ g entering the capillary tube.

The majority of the peaks that were obtained from the uncoated tubes possessed a very steep leading edge. With some surfaces, and for some substances, the peaks were quite narrow and symmetrical, but more often tailing was encountered. Exceptionally (and only at the highest concentration level), peaks having a degree of "leading" were observed.

For the purposes of a direct comparison of the individual cleaning steps and for simple evaluation of the surface activity, the chromatographed substances were characterized by their capacity ratios (*k*) and peak profiles. The peak profiles were evaluated quantitatively using the asymmetry factor¹⁹ $F_t = 100 (a/b)$, where *a* is the distance between the leading peak boundary and the perpendicular drawn from the peak maximum to the baseline, measured at 10% of the peak height, and *b* is the corresponding distance between the perpendicular line and the trailing boundary.

RESULTS AND DISCUSSION

This work summarizes a large number of results obtained on more than 20 capillary tubes. In the course of the experiments, large differences in the retention activities of individual capillaries were observed, but it was not possible to correlate them with the origin of the tubing in any statistically significant manner. Indeed, within each of the three groups of samples received from the manufacturers cited (and representing, with regard to mechanical properties and wall thickness, three different types of material), approximately the same range of adsorption behaviour was observed as in all of the samples. For this reason, the particular tubes will not be distinguished with respect to their origin.

The efficiency of different cleaning methods for new tubing

The walls of a new, otherwise untreated, tube very strongly retain the vapours

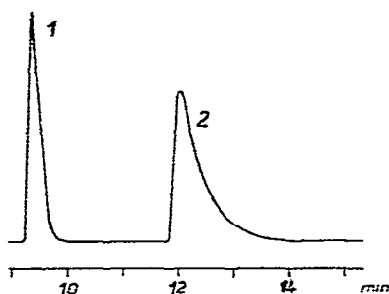


Fig. 1. Gas chromatography of *n*-alkanes on a stainless-steel capillary which was tested as received. Tube D-0 (50 m \times 0.25 mm I.D.); temperature, 60°; inlet pressure, 0.51 bar; sample size, 0.10 μ l (splitting ratio, 1:1000). Peaks: 1 = *n*-pentane; 2 = *n*-hexane.

from the carrier gas. Injections of polar compounds gave no detector response. In general, only the lightest alkanes and cycloalkanes, tetrachloromethane, and, in some instances, benzene were able to pass through the column and give a measurable response; even then they appeared as rather distorted peaks. Fig. 1 shows a typical example of the chromatographic behaviour of a tube tested as received from the manufacturer: *n*-pentane and *n*-hexane were eluted as resolved but tailing peaks, whereas *n*-heptane (contained in the sample) did not appear on the chromatogram. With the most retentive tubes, even pentane was extremely retarded under comparable conditions.

TABLE I

CAPACITY RATIOS AND ASYMMETRY FACTORS FOR SUBSTANCES CHROMATOGRAPHED ON AN "EMPTY" STAINLESS-STEEL CAPILLARY AFTER SUCCESSIVE CLEANING STEPS

Temperature, 60°; sample size, 0.05 μ l; splitting ratio, 1:1000. Tube D: surface D-0, as supplied; D-1, after one cleaning with organic solvents; D-2, after two cleanings with organic solvents; D-4, after three treatments with organic solvents and one treatment involving HNO₃ and NH₄OH; D-5, after repeated treatment with HNO₃ and NH₄OH. Substances which were not eluted at all are denoted by N.

Compound	D-0		D-1		D-2		D-4		D-5	
	<i>k</i>	<i>F_t</i>	<i>k</i>	<i>F_t</i>	<i>k</i>	<i>F_t</i>	<i>k</i>	<i>F_t</i>	<i>k</i>	<i>F_t</i>
<i>n</i> -Hexane	0.47	35	0.02	100	0.02	100	0.02	100	0.01	100
<i>n</i> -Octane	N	N	0.20	33	0.20	35	0.12	66	0.05	100
<i>n</i> -Decane	N	N	1.91	2	1.75	3	0.82	5	0.29	70
<i>n</i> -Dodecane	N	N	N	N	N	N	N	N	1.61	9
Benzene	3.0	<1	0.11	40	0.09	35	0.05	70	0.02	100
Toluene	N	N	0.39	4	0.30	10	0.14	26	0.04	100
Ethylbenzene	N	N	1.16	2	0.91	8	0.37	6	0.09	80
Diethyl ether	N	N	0.21	11	0.15	15	0.09	24	0.01	100
Acetone	N	N	N	N	0.61	<1	0.21	4	0.03	90
Methanol	N	N	N	N	N	N	N	N	0.09	5
Pyridine	N	N	N	N	N	N	N	N	N	N
Nitromethane	N	N	0.27	3	0.20	3	0.17	6	0.03	60
Ethyl acetate	N	N	N	N	0.74	<1	0.70	<1	0.04	75
Tetrachloromethane	0.60	20	0.03	100	0.03	100	0.03	100	0.01	100

The cleaning of the walls was reflected distinctly in shortened retention times and improved peak shapes. As an illustration, the progress of cleaning of a typical capillary is documented in Table I in terms of the capacity ratios and asymmetry factors of the substances chosen. Only the results obtained for the 0.05- μ l sample size are given here; the concentration dependence followed a general pattern in that it was strong for the more retarded compounds (the capacity ratios being affected more than the F_i factors and decreasing with increasing concentration, *e.g.*, from 1.5 to 0.6) and markedly diminished with the overall shortening of retention times. It can be seen, that the first two cleaning steps, conducted with the standardized series of organic solvents, caused a substantial reduction in the retention activity (surfaces D-1 and D-2) compared to that of the original surface (D-0). When this method of cleaning was repeated, the results were substantially unaltered. (Data for D-3 are not tabulated for this reason.) A further change, however, was effected by flushing with nitric acid and ammonia (procedure b). The final surface, D-5, is characterized by a very low adsorptivity; in this case, the k and F_i values were almost constant with respect to sample size. The remarkable ability of this surface to resolve homologous hydrocarbons is illustrated in Fig. 2. The separation of aromatics (Fig. 2b) may be compared with an analogous chromatogram obtained on a silver-plated steel capillary, destined specifically for gas-solid chromatography (GSC)²⁰.

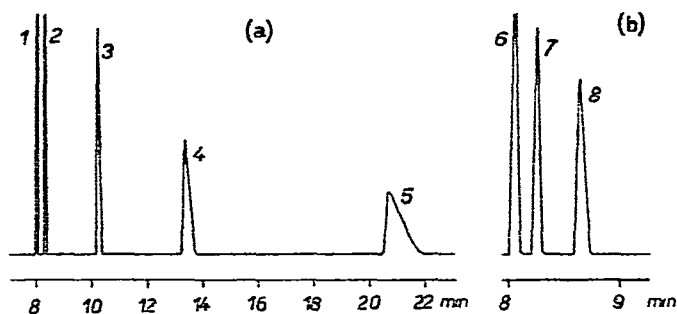


Fig. 2. Separation of *n*-alkanes (a) and of aromatic hydrocarbons (b) by GSC on a thoroughly cleaned stainless-steel capillary. Tube D-5. Conditions as in Fig. 1, sample size, 0.25 μ l (a) and 0.15 μ l (b). Peaks: 1 = *n*-hexane; 2 = *n*-octane; 3 = *n*-decane; 4 = *n*-undecane; 5 = *n*-dodecane; 6 = benzene; 7 = toluene; 8 = ethylbenzene.

The overall experience gained can be summarized as follows.

(1) The reproducibility of the behaviour of individual tubes within a group was very poor during cleaning. With some of the capillaries, the reduction in surface activity was quite slow, while other capillaries were easy to clean. The magnitude of the primary retention activity seemed to give no indication of its future behaviour.

(2) Nevertheless, the results of cleaning are fairly reproducible. By carefully controlled cleaning, low adsorptivity parameters comparable to those of the surface D-5 could be achieved for most tubes. This was accomplished either by repeated washings with organic solvents or by treatment with nitric acid and ammonia. The latter method required less time and also worked in cases where the former method failed (as in the example of tube D). For some tubes, however, the retentivity could not be lowered substantially by any means.

(3) While, at least in certain cases, surfaces with similar characteristics could be obtained by means of methods a and b, the remaining methods of cleaning (c, d and e) obviously differed. When low-adsorptivity tubes, prepared according to method b, were treated according to these methods, the adsorption affinity increased, especially with the methods involving ammonia and Komplexon. This behaviour is different from that of new (or only partially cleaned) tubing, as higher-boiling and more polar compounds were eluted reproducibly. This observation appears to indicate some chemical modifications of the walls.

(4) It was not possible to decrease the adsorptivity beyond the level corresponding to that of the tube D-5 (Table I). Obviously, this represents a limiting state, at least with the types of tubing investigated. The question remains whether such surfaces can be regarded as truly "clean", but their properties are well suited as a reference basis for comparative purposes. Accordingly, a "low-adsorptivity surface" may be defined and quantitatively characterized by the values of k and F_i respectively. Both quantities are related to the adsorption isotherm for a particular substance, the k value, at a very low concentration level, being a measure of the initial slope of the isotherm, and the shape of the peak being determined by its further course. For "inert" surfaces, the capacity ratios should be very small, and with linear isotherms the peaks should be symmetrical.

Regeneration of used open tubular columns

As with new tubing, the chromatographic test is a useful means of following the purging of an old liquid-coated open tubular column before regeneration. Retention behaviour intermediate between that of a "fully" coated column and that of an uncoated tube was usually observed after the first simple cleaning procedure had been performed. This behaviour was characterized by comparatively high k values and good peak symmetry for the less volatile solutes, and indicates that some residues of the stationary phase are much more difficult to remove than the bulk of the layer. With repeated washings, the original adsorption characteristic of the walls could be restored in almost every case, however; again, cleaning with nitric acid and ammonia proved to be most efficacious, but in several instances cleaning with organic solvents was also sufficient. The ease with which the deposited phase was removed from the individual columns is probably dependent on the previous history of column use (actions of heat, air, etc.); of course, such relations are very difficult to confirm.

Relation between the surface adsorptivity and the performance of liquid-coated open tubular columns

Several capillaries with previously determined surface activities were chosen for coating with squalane. The attainment of the maximum separation efficiency was not attempted, but, rather, columns were obtained with approximately equal amounts of the stationary phase. For *n*-nonane at 60°, the capacity ratios ranged from 8.8 to 13.1, and the efficiencies were 90,000–150,000 theoretical plates.

The k and F_i values of the tubes used, measured prior to coating, are summarized in Table II. Tubes A, C and H belong to the "low-adsorptivity" group; there are only minor differences among them (and when compared to the tube D-5). Tube Q is an example of a surface that retained a high degree of activity even after exhaustive treatments by the methods a and b. Tubes B, F and K are representative of the

TABLE II

ADSORPTION CHARACTERISTICS OF SURFACES OF CAPILLARY TUBES USED IN THE PREPARATION OF SQUALANE-COATED COLUMNS

Temperature, 60°; sample size, 0.05 μ l; splitting ratio, 1:1000. For other details see text.

Compound	A		C		H		Q		B		F		K	
	<i>k</i>	<i>F_t</i>	<i>k</i>	<i>F_t</i>	<i>k</i>	<i>F_t</i>	<i>k</i>	<i>F_t</i>	<i>k</i>	<i>F_t</i>	<i>k</i>	<i>F_t</i>	<i>k</i>	<i>F_t</i>
<i>n</i> -Hexane	0.01	100	0.01	100	0.01	100	0.10	100	0.03	100	0.08	33	0.03	100
<i>n</i> -Octane	0.06	90	0.07	90	0.06	100	0.53	33	0.11	90	1.21	11	0.28	15
<i>n</i> -Decane	0.35	33	0.44	11	0.36	30	3.03	20	0.66	10	9.5	<1	2.82	<1
<i>n</i> -Dodecane	2.10	3	2.80	5	2.40	12	N	N	4.2	<1	N	N	N	N
Benzene	0.02	95	0.02	95	0.02	100	0.33	20	0.04	95	0.10	16	0.08	33
Toluene	0.04	90	0.06	60	0.05	80	0.84	10	0.10	33	0.40	12	0.24	9
Ethylbenzene	0.10	40	0.12	20	0.11	40	1.75	8	0.25	8	1.08	8	0.72	4
Diethyl ether	0.01	95	0.01	100	0.02	100	0.12	60	0.13	9	0.06	50	0.10	16
Acetone	0.01	90	0.05	15	0.07	25	0.40	6	0.42	<1	0.16	7	0.39	3
Methanol	0.04	18	0.33	3	0.11	5	0.80	3	N	N	N	N	N	N
Pyridine	0.12	<1	2.5	<1	N	N	N	N	N	N	N	N	N	N
Nitromethane	0.04	40	0.03	22	0.05	50	0.67	5	0.14	10	0.25	5	0.42	<1
Ethyl acetate	0.02	80	0.11	10	0.13	10	0.49	6	0.76	<1	0.41	6	1.26	<1
Tetrachloromethane	0.01	90	0.01	100	0.02	100	0.23	80	0.02	95	0.07	90	0.04	90

results obtained by the cleaning methods c, d and e, respectively; they were prepared by repeating these treatments until the adsorptivity was constant.

The differences in the GC behaviour of the individual squalane-coated columns can be followed by comparing the retention indices of substances of varying polarity (Table III) and the peak-asymmetry factors of oxygen-containing compounds (Table IV). The agreement between columns is very satisfactory, even for polar compounds, with the tubes A, C and H. On the other hand, exceptionally high retention indices, together with a striking tendency to tailing, are characteristic of column Q; this applies to a lesser extent to column F. No such clear correlation exists between the adsorption properties of the clean surface and the performance of the coated column

TABLE III

RETENTION INDICES OF VARIOUS SUBSTANCES AT 60° ON SQUALANE-COATED, STAINLESS-STEEL, OPEN TUBULAR COLUMNS WITH CONTROLLED SURFACE ADSORPTIVITY

Compound	A	C	H	Q	B	F	K
2-Methyloctane	864.6	864.7	864.6	864.6	864.6	864.6	864.7
2,3,5-Trimethylhexane	812.9	812.8	812.9	812.7	812.9	812.8	813.0
Methylcyclohexane	728.0	727.8	728.0	730.3	728.0	728.0	728.2
1-Octene	781.8	781.7	781.7	783.0	781.7	782.1	782.0
Toluene	748.9	748.8	748.7	759.8	747.9	750.0	749.1
<i>o</i> -Xylene	873.8	873.8	873.9	885.3	872.9	875.3	874.1
<i>n</i> -Butyl bromide	705.5	705.5	705.8	715.0	704.8	706.8	705.8
Chlorobenzene	816.5	817.0	817.1	847.6	815.7	818.2	816.1
Thiophene	644.5	644.6	644.5	661.7	645.1	645.2	643.4
Anisole	871.8	872.2	872.0	892.7	870.1	874.4	871.2
<i>n</i> -Butyl acetate	752.6	752.0	751.3	769.1	748.7	756.6	751.5
2-Hexanone	734.0	731.7	731.5	761.3	728.3	736.1	731.3

TABLE IV

ASYMMETRY FACTORS, F_s , FOR OXYGEN-CONTAINING COMPOUNDS ON SQUALANE-COATED, STAINLESS-STEEL, OPEN TUBULAR COLUMNS WITH CONTROLLED SURFACE ADSORPTIVITY

Compound	A	C	H	Q	B	F	K
Anisole	100	100	100	60	100	90	100
<i>n</i> -Butyl acetate	100	100	100	100	100	100	100
2-Hexanone	100	100	100	50	100	90	100
<i>p</i> -Dioxane	100	90	100	60	100	80	100
Ethanol	60	10	16	<1	30	30	45

with capillaries B and K, however. It can only be supposed that these two surfaces, prepared with the aid of nitric acid and Komplexon, respectively, possess a qualitatively different nature and that the active sites are more readily covered by the stationary phase.

For all types of hydrocarbons, as well as for halogen-containing hydrocarbons, the peaks obtained were perfectly symmetrical on all of the columns. Deviations were encountered only for the more polar, oxygen-containing, compounds (Table IV). The best symmetry for ethanol was obtained on column A, which, in the uncoated state, also had the least affinity for methanol. The performance of this column is illustrated by the chromatogram in Fig. 3, showing the resolution of the so-called "polarity mixture". To my knowledge, this is the only case in which such a good symmetry for the alcohol peak has been obtained on a squalane-coated steel open tubular column without the addition of any surface-active material (*cf.* ref. 21).

Some further observations can be made concerning the temperature stability of the columns. The results given so far were obtained on columns for which the conditioning temperature did not exceed the temperature of the measurements (60°). After having been aged at 100° for 24 h, the columns were retested at 60°. Columns A, C, H and K were stable under these conditions, neither the retention indices nor

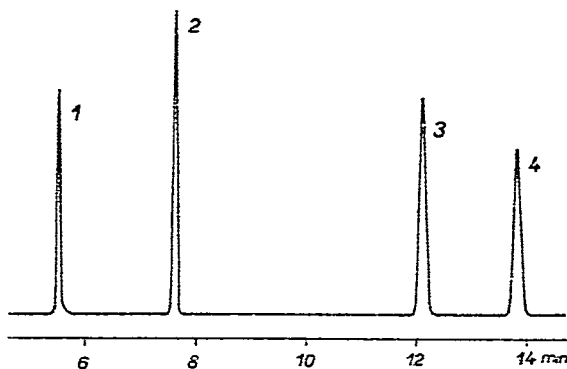


Fig. 3. Resolution of the polarity mixture on a squalane-coated stainless-steel capillary with low surface adsorptivity. Column A (see text) (50 m × 0.25 mm I.D.); temperature, 60°; inlet pressure, 0.84 bar; sample size 0.10 μ l (splitting ratio, 1:1000). Peaks: 1 = ethanol; 2 = ethyl methyl ketone; 3 = benzene; 4 = cyclohexane.

the separation ability being affected. With the other columns, the retention indices increased by various extents (with the exception of those of alkanes and olefins).

CONCLUSIONS

It would not be appropriate to generalize the results of this study to all of the possible types of stainless-steel tubing that are used in the preparation of open tubular columns. More information would be needed about the influences of varying chemical composition and mechanical surface structure. Nevertheless, the following conclusions seem to be justified.

(1) It is very useful to follow the progress of cleaning by means of GC. The knowledge of the adsorption activity of the surface prior to coating is important in determining the final performance of the coated open tubular column.

(2) By means of carefully controlled cleaning, stainless-steel surfaces can be prepared which possess low adsorptivity. Until a better understanding is gained of the relations between simple cleaning and chemical modification, the low-adsorptivity surface defined here and characterized by capacity ratios (at 60°) of $k \leq 0.45$ for *n*-decane, $k \leq 0.12$ for ethylbenzene, $k \leq 0.08$ for acetone and $k \leq 0.02$ for diethyl ether, as well as by the good peak symmetry for these compounds, can be taken as a suitable reference basis.

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