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EVALUATION OF CAPILLARY GAS CHROMATOGRAPHIC COLUMNS IN SERIES

ANALYTICAL APPLICATION TO LEMON OIL

G. PAOLO CARTONI*, GIANCARLO GORETTI, BENNARDO MONTICELLI and MARIO VINCENZO RUSSO
Dipartimento di Chimica, Università "La Sapienza", P. le Aldo Moro 5, 00185 Rome (Italy)
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SUMMARY

The advantages of capillary columns connected in series for solving analytical problems are discussed. Two columns of the same length and very different polarity (SE-54 and PEG 20M) were used. Under isothermal conditions, owing to the carrier gas compressibility a noticeable increase in polarity is observed when the sequence of columns SE-54–PEG 20M is reversed. With temperature programming, an increase in the programming rate decreases the influence of the second column. By changing the programming rate during the analysis it is possible to optimize the column polarity to obtain the best separations. An application to the analysis of lemon oil is reported; complete separation of the most important components was achieved.

INTRODUCTION

The analysis of very complex mixtures by gas chromatography is still a problem because, in spite of the high efficiency achieved with capillary columns, it is not always possible to obtain a complete separation in a single column. To solve this problem different approaches have been tried: (1) parallel columns with a single injector and multiple detection; (2) mixed phase columns; and (3) columns connected in series.

For the first method it is necessary to have different detectors and recorders and to carry out a complicated data analysis. The second method is limited to the use of miscible liquid phases and only one polarity is obtained for each column. In the third method it is possible to use a single injector and detector, and the polarity of the coupled columns can also be changed.

Packed gas chromatographic columns have been connected in series to solve some specific problems[1–5] and more recently capillary columns have been used. Pretorius and Smuts[6] and Kaiser and Rieder[7] developed a procedure based on connecting in series columns of the same length, with independent temperature control for each.

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A general theory was developed by Purnell and co-workers on the use of these columns under isothermal conditions[8] and they investigated the resulting efficiency[9]. Kaiser *et al.*[10] presented a method based on the use of a pressure regulator placed between two different columns; by changing their flow-rates it is possible to change the polarity of the system.

In this work a system obtained by connecting in series two columns of very different polarity (SE-54 and PEG 20M) was studied and the effect of changing the temperature programme was investigated. The method has been applied to the analysis of lemon oil and a complete separation of the most important compounds was achieved, whereas this was not possible with any single column[11].

EXPERIMENTAL

Two glass capillary columns of the same length and diameter (15 m × 0.20 mm I.D.) were coated with PEG 20M as described elsewhere[12] and with SE-54 as reported by Grob[13]. The stationary phases were cross-linked and hence could be solvent washed and were more stable during temperature programming.

The two columns were connected in series either with PTFE tubing or with a Supelco butt-connector (Cat. No. 2-3796), taking care to avoid any dead volume. The columns were tested by injections of a standard mixture containing *n*-octanol (Oct), 2,6-dimethylaniline (DMA), methyl decanoate (E_{10}), phenol (Ph), *o*-, *m*- and *p*-cresol (Cres), 2,6-dimethylphenol (DMF) and some *n*-alkanes from C_9 to C_{22}.

The columns were tested separately and also connected in different sequences. The measurements were carried out under various isothermal conditions in the range from 90 to 150°C and with temperature programming from 80 to 180°C at rates of 0.5, 1.0, 2.0, 4.0, 8.0 and 12.0°C/min. The temperature programming rate (TPR) was also changed in the middle of the analysis time by using first a low rate followed by a high rate, or *vice versa*.

The analysis of lemon oil was carried out with the single columns and with the columns in series, optimizing the temperature programming rate.

A Hewlett-Packard Model 5890 gas chromatograph equipped with a flame ionization detector and a Shimadzu Model CR-34 integrator was used with hydrogen as the carried gas.

All chemical used were obtained from Carlo Erba (Milan, Italy) and the lemon oil was a commercial product from Sicily.

RESULTS AND DISCUSSION

The efficiency of the columns alone and connected in series was measured with a standard mixture, at 100°C, of tetradecane, 2,6-dimethylaniline and methyl decanoate. The results, reported in Table I, show that the number of theoretical plates of the two columns connected in series is about 80% of the sum of the values for the individual columns at the same linear gas velocity (44 cm/s).

The best efficiency is obtained for methyl decanoate, which has similar values of the capacity ratio (k') for both columns. The values of k' for the standard mixture *versus* column fraction are shown in Fig. 1. The values for the two columns connected in series are not exactly 0.5 for the effect of the carrier gas compressibility. The

TABLE I

COLUMN EFFICIENCY FOR THE TWO DIFFERENT COLUMNS AND THEIR COMBINATION IN SERIES FOR SOME REFERENCE COMPOUNDS

Compound	*SE-54*		*SE-54–PEG 20M*		*PEG 20M–SE-54*		*PEG 20M*	
	k'	*n*	*k'*	*n*	*k'*	*n*	*k'*	*n*
DMA	4.06	64 800	11.32	100 000	14.15	102 000	21.04	60 500
C_{14}	17.56	69 000	10.80	95 000	8.60	99 000	2.10	65 000
E_{10}	10.96	65 700	8.74	115 000	8.16	120 000	6.13	60 200

contribution of PEG 20M is 0.45 with the columns in the order SE-54–PEG 20M and 0.58 for the order PEG 20M–SE-54.

It is interesting that, with the large difference in polarity between these two columns, the most retained compounds in one column are the least retained in the other, and consequently there are no great increases in the total analysis times when using the two columns connected in series. The retention indices (RI) measured for some test substances on the SE-54 column in the temperature range 90–140°C are reported in Table II. Table II also gives the ΔI values relative to the SE-54 column

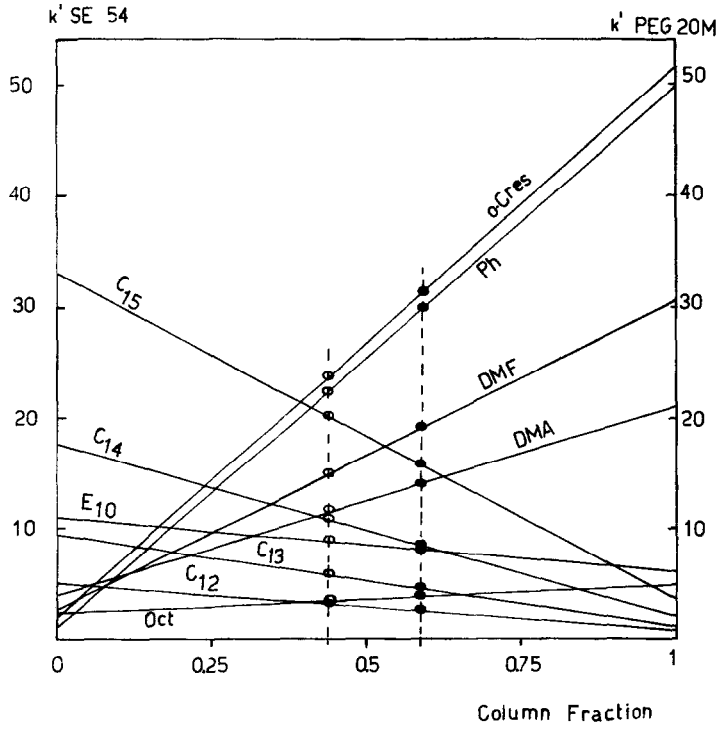

Fig. 1. *k'* values of standard compounds *versus* column fraction with columns in different orders: ○, SE-54–PEG 20M; ●, PEG 20M–SE-54.

TABLE II

RETENTION INDICES (RI) ON AN SE-54 COLUMN AND THEIR INCREMENTS (ΔI) ON THE OTHER COLUMNS

Temperature (°C)	*RI (SE-54)*					*ΔI (SE-54–PEG 20M)*					*ΔI (PEG 20M–SE-54)*					*ΔI (PEG 20M)*				
	Oct	E_{10}	*DMA*	*Ph*	*m-Cres*	*Oct*	E_{10}	*DMA*	*Ph*	*m-Cres*	*Oct*	E_{10}	*DMA*	*Ph*	*m-Cres*	*Oct*	E_{10}	*DMA*	*Ph*	*m-Cres*
90	1070	1327	1167	976	1075	144	36	236	538	510	200	60	305	624	595	478	220	628	878	955
100	1071	1326	1171	975	1072	143	40	236	538	510	200	65	308	625	593	479	235	633	983	962
110	1072	1325	1172	974	1072	143	43	239	536	509	200	70	313	624	599	478	267	642	991	971
120	1072	1324	1170	974	1072	141	46	236	535	508	200	75	312	622	600	478	269	644	999	986
130	1072	1324	1184	975	1072	140	48	236	532	506	201	75	315	620	602	479	276	648	1005	987
140	1073	1323	1190	974	1073	140	51	236	530	504	202	81	318	619	602	478	277	653	1011	992

for the columns connected in series in the two different orders and for the PEG 20M column alone.

The polarity, as shown, increases in the order SE-54, SE-54–PEG 20M, PEG 20M–SE-54, PEG 20M. With temperature programming the SE-54 column does not show a noticeable variation of RI values with the programming rate, and the PEG 20M column shows only small increments. With the columns connected in series there is, as expected, a large effect, as shown in Fig. 2. With the columns in the order SE-54–PEG 20M the RI decreases with TPR, whereas in the order PEG 20M–SE-54 it increases. This opposite behaviour is explained by the fact that the compounds

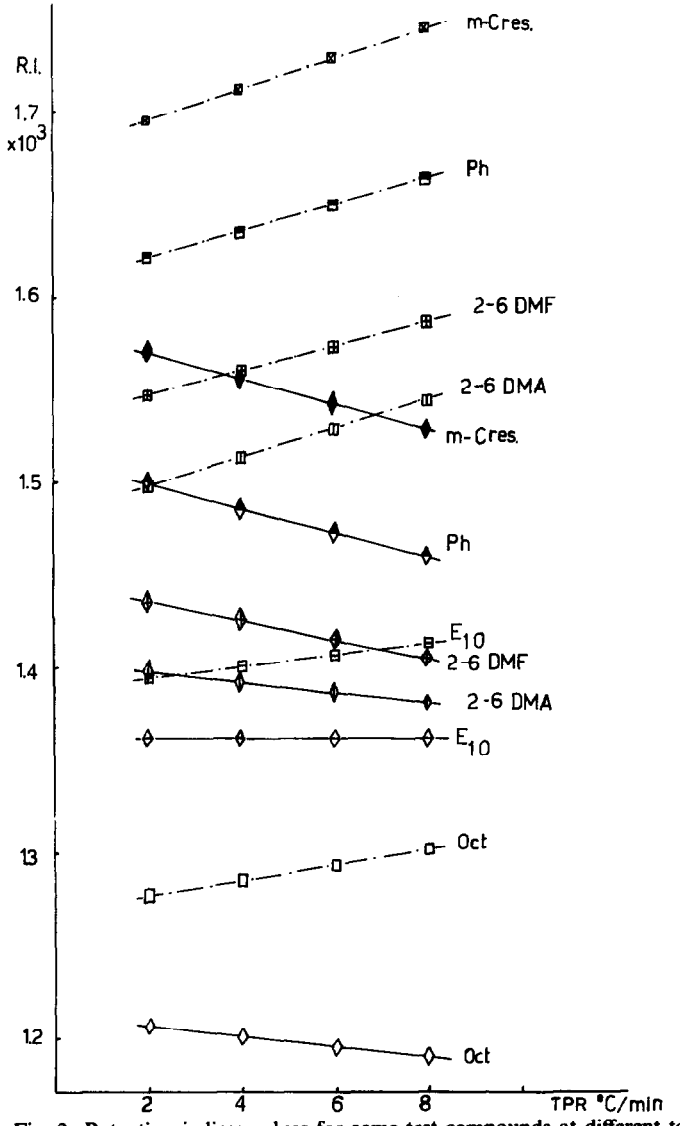

Fig. 2. Retention indices values for some test compounds at different temperature programming rates (– · – · –), PEG 20M–SE-54; (———), SE-54–PEG 20M.

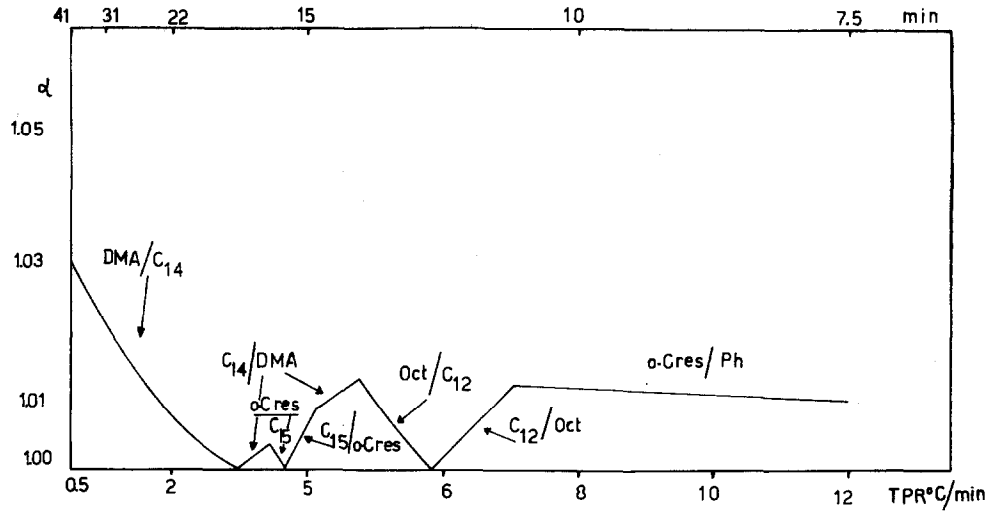

Fig. 3. Plots of separation factor (α) versus temperature programming rate. Column order: SE-54–PEG 20M.

enter the second column at higher temperatures than the first, and consequently the influence of the second column decreases. The system becomes more and more polar on increasing the TPR in the order PEG 20M–SE-54, and less and less polar in the opposite order SE-54–PEG 20M.

Fig. 3 shows a "window" diagram obtained by plotting the separation factor,

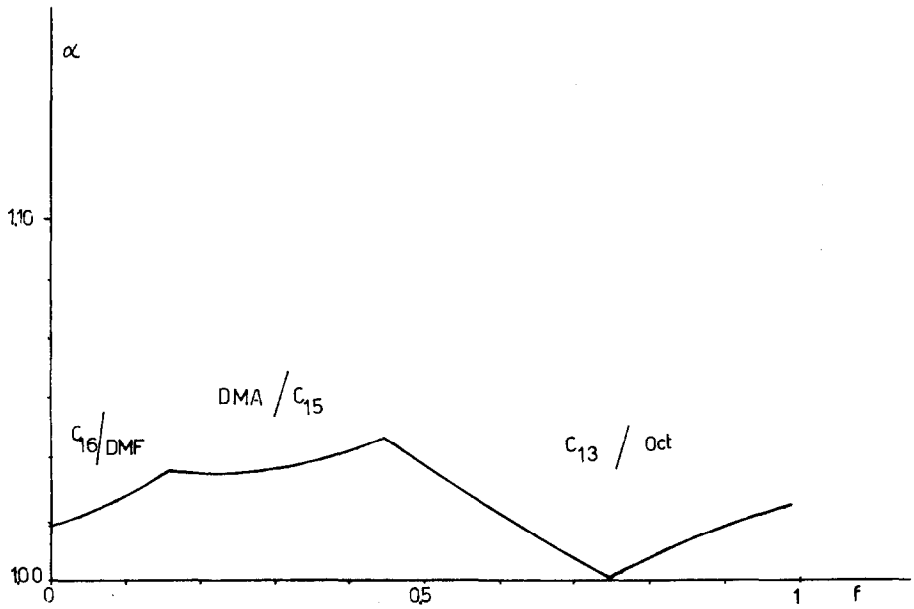

Fig. 4. Plots of separation factor (α) versus temperature increase ratio ($\Delta T_1/\Delta T$). Column order: PEG 20M–SE-54.

α, against log TPR for some pairs that are difficult to separate. As can be seen, there are two different programming rates (at about 5 and 7°C) where good separations are achieved, but as we consider also the analysis time reported in the upper scale, the second TPR is to be preferred, because almost the same separations are obtained but in a shorter time.

The polarity of the system obtained by connecting the columns in series can also be changed if two temperature programming rates are used. In this instance, by using first a high TPR and then a low TPR, or *vice versa*, the polarity changes, but the analysis time remains almost constant. Fig. 4 shows a "window" diagram for the PEG 20M–SE-54 system obtained by changing the TPR, always at the same time (8.25 min), which corresponds to half of the analysis time. The separation factor for some pairs is plotted against the ratio $\Delta T_1/\Delta T$, which is the ratio between the temperature increment from the beginning to the half-time and the temperature increment from the beginning to the end.

In this way the ratio goes from 0 (isothermal conditions for the first part and a rate of 10°C/min for the second part), to 1 (isothermal temperature in the second part after an increase at 10°C/min in the first part). The column polarity changes, and with $\Delta T_1/\Delta T < 0.5$ for compounds eluted before the middle of the chromatogram, the second apolar column (SE-54) is the more important because it is still at

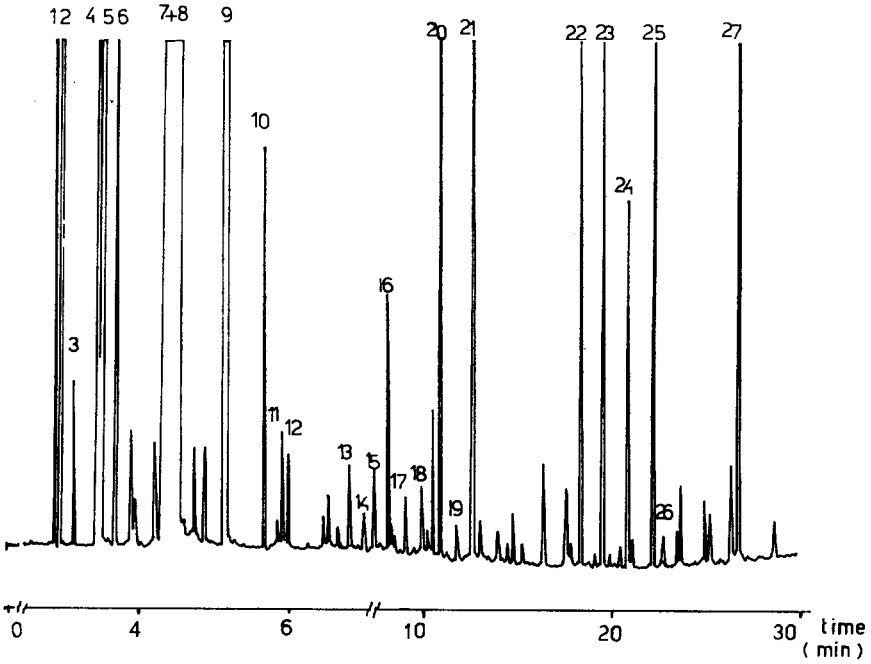

Fig. 5. Chromatogram of a lemon essential oil on an SE-54 column. $\bar{U}$ = 45 cm/s; T = 60°C for 5 min, then programmed at 2°C/min to the end. Compounds: 1 = α-thyjene; 2 = α-pinene; 3 = camphene; 4 = sabinene; 5 = β-pinene; 6 = myrcene; 7 = limonene; 8 = p-cymene; 9 = γ-terpinene; 10 = terpinolene; 11 = linalool; 12 = nonanal; 13 = citronellal; 14 = decanal; 15 = 4-terpinenol; 16 = α-terpineol; 17 = citronellol; 18 = nerol; 19 = geraniol; 20 = neral; 21 = geranial; 22 = neryl acetate; 23 = geranyl acetate; 24 = caryophyllene; 25 = bergamotene; 26 = humulene; 27 = β-bisabolene.

low temperature, whereas for late-eluted compounds the second column becomes less important because its temperature rapidly increases.

An opposite behaviour occurs with $\Delta T_1/\Delta T > 0.5$ and, of course, also on changing the column sequence. In this way many different situations can be obtained and adapted to any particular analysis.

Analysis of an essential oil

Among the many possible applications of the described system, we analysed a lemon oil. Because of the large number of components of different polarity, it is very useful to have the facility to change the column polarity to obtain the best separation.

Figs. 5 and 6 show the best chromatograms obtained on the single columns. With SE-54 the pair limonene–*p*-cimene is unresolved and a very little separation is achieved for sabinene–β-pinene. With PEG 20M alone there is a good separation of many compounds but the pair α-thujene–α-pinene is not resolved at all, and a very poor resolution is obtained for geranial–β-bisabolene.

With the columns connected in series, after some attempts, a complete separation for all the major components was achieved with the columns in the order PEG 20M–SE-54, as shown in Fig. 7. The working conditions reported give a good separation in a short analysis time. By changing the order of the columns to SE 54–PEG 20M no complete separation could be obtained using any temperature programme.

Hence by connecting two capillary columns of different polarity in series, a column system of tunable polarity can be obtained simply by changing the temper-

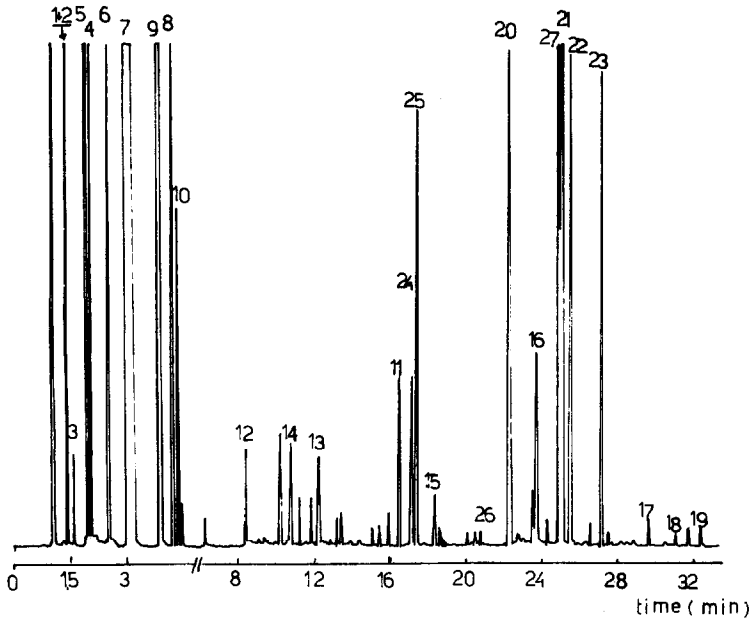

Fig. 6. Chromatogram of the same lemon oil as in Fig. 5 on a PEG 20M column under the same operating conditions. Peaks as in Fig. 5.

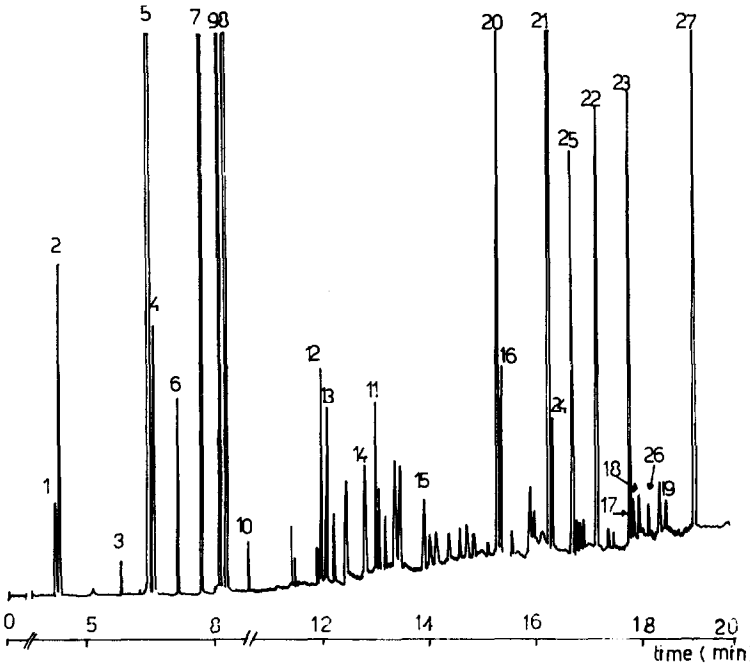

Fig. 7. Chromatogram of the same lemon oil as in Fig. 5 on a PEG 20M–SE-54 column system. Temperature programmed from 35 to 90°C at 5°C/min, then from 90°C to the end at 2°C/min. Peaks as in Fig. 5.

ature programming rates. This column system can easily be installed in any commercial apparatus without any modification, and it gives a very versatile system that can be applied to the analysis of many complex samples.

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