TERPENOIDS

CXX. EVALUATION AND COMPARISON OF INDIAN FIRE-BRICK AS A SUPPORT WITH CELITE IN GLC COLUMNS*

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Gas–liquid chromatography (GLC) has proved to be an important analytical tool for analyses of natural products. In this technique, the column plays an important role, the efficiency of it depends not only on the liquid substrate but the solid support also plays a critical part[1].

We have been using this technique for quite a long time for the analyses of terpenes and allied products and, so far, have systematically studied series of polyesters, derived from various glycols and dicarboxylic acids etc. with very interesting results[2-5].

During these investigations, because of the non-availability of imported supporting materials, such as Chromosorbs etc. we have had to depend mostly on locally available fire-brick powder and this has been successfully used as a support for GLC columns.

As it is constantly used in our laboratory, it was felt that a proper evaluation of this Indian fire-brick as a column support was essential and hence its thorough study was undertaken. Celite 545 has been used all along for a systematic comparison and some of the results are incorporated in this communication.

EXPERIMENTAL

A modified Griffin and George Model MK II VPC apparatus, has been used, as described in our earlier communication[2]. The conditions were:

Temperature 170° ($\pm$ 1°) (no injection port heating).

Flow rate: 4 l/h, of hydrogen.

Column: 6 mm I.D.; 2 m long.

Stationary phase: sebacic acid–diethylene glycol polyester; prepared in our laboratory[2].

Preparation and general properties of the fire-brick powder

Several varieties of fire-brick were investigated. Some of them definitely proved to be catalytically active while a few were found to be too porous. Finally a

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variety that had a surface area of nearly 2 sq. m/g was selected and used. The crushed material was placed in a 5 l flask and washed a number of times with tap water. During this step it was possible to remove fine particles. The same material was treated with conc. hydrochloric acid for 24 h and was repeatedly washed with water until free from acid. It was then treated with hot concentrated alkali and allowed to stand overnight, washed with distilled water till free from alkali and allowed to drain and finally ignited at about 600° for several hours, and then transferred to a pyrex glass container and cooled. It was stored in an air tight container. The storage of such material in an air tight container has proved very useful. Similar observations have been reported earlier[6]. It is white in colour and hard enough not to give any fine powder during treatment or while packing in the columns.

Packing density of the fire-brick powder

A 25 ml burette, calibrated at 20° was used for this purpose. Fire-brick powder (120–150 mesh) was packed into the burette, in the same way as that used for packing columns. An average of five observations gave its density to be 0.78 g/ml.

Impregnation

This process was carried out as described previously[2]. Sebacic acid–diethylene glycol polyester was used throughout as the stationary phase. The following percentages (w/w) of stationary phase were used: (1) 1.0 %, (2) 3.0 %, (3) 5.75 %, (4) 9.0 %, and (5) 15.2 %.

For direct comparison, Celite 545 (120–150 mesh) was also used as the column packing. This material was purified as in the case of fire-brick powder. Two columns were prepared, one with 15 % (w/w), the other with 20 % stationary phase.

Filling the columns

A fixed volume of the packing material was slowly added to the column, the column being continuously tapped with a rubber hammer, until the packing material had completely settled down.

Finally the column ends were closed with glass wool plugs. As the weight of the total column filling had been noted, it was possible to estimate the actual weight of the stationary phase in a particular column.

Filling the Celite 545 columns

Even though the percentage weight of the stationary phase differed in the case of both the columns, the total weight of the column packing was adjusted in such a way, that both the columns had nearly the same weight of stationary phase.

Samples

The following terpenic samples were employed for the comparative evaluation of packing material.

Hydrocarbons. (1) Longicyclene; (2) longifolene; (3) humulene; (4) caryophyllene.

Carbonyl compounds. (5) Carvone and (6) camphor.

Alcohol. (7) Borneol.

Semi-aromatic compounds. (8) Dihydro ar-curcumene and (9) ar-himachalene.

TABLE I

EFFECT OF LIQUID LOADING ON RETENTION TIME AND BAND BROADENING

Compound	Percentage of stationary phase on fire-brick support (120–150 mesh)								Percentage of stationary phase on Celite 545					
	1%		3%		5.75%		9%		15.2%		15%		20%	
	Peak width (mm)	t_R (mm)	Peak width (mm)	t_R (mm)	Peak width (mm)	t_R (mm)	Peak width (mm)	t_R (mm)	Peak width (mm)	t_R (mm)	Peak width (mm)	t_R (mm)	Peak width (mm)	t_R (mm)
Longicyclene	3.4	14.1	5.0	29.5	6.5	43.5	10.0	70.5	9.0	47.5	10.0	67.5	8.0	49.5
Longifolene	3.0	15.8	6.0	36.0	8.0	55.5	12.5	86.5	9.5	60.0	11.5	83.5	10.0	62.5
Humulene	3.5	21.0	7.5	44.5	9.5	73.5	15.5	121.5	7.0	42.0	15.5	115.5	13.0	87.5
Carvone	3.7	17.5	6.5	43.5	9.0	67.0	14.0	112.5	12.5	90.0	14.0	103.5	11.5	84.2
Caryophyllene	4.6	17.2	8.0	40.0	8.5	58.5	13.0	94.5	16.0	75.0	13.5	89.5	10.0	68.5
Camphor	2.0	11.0	4.0	24.5	5.0	35.5	9.0	59.0	12.5	83.0	8.5	54.5	—	—
Borneol	2.5	14.0	5.0	34.5	7.5	52.5	11.5	85.0	—	—	12.0	80.5	9.5	66.6
Dihydroar-curcumene	6.5	20.8	—	—	9.0	66.5	14.8	104.0	12.0	85.0	16.0	99.5	11.0	75.5
Ar-himachalene	7.2	34.4	—	—	16.5	135.5	25.5	218.5	22.5	181.0	28.5	206.5	23.5	162.5

Recorder chart speed 12 in./h   Recorder chart speed 6 in./h   Recorder chart speed 12 in./h

TABLE II

COMPARISON OF COLUMN PERFORMANCE: VALUES OF 'S_{12}', 'Q' AND 'R' FOR THE COMPOUND PAIR LONGICYCLENE AND CARVONE

Percentage of stationary phase on fire-brick support												Percentage of stationary phase on Celite 545								
1%			3%			5.75%			9.0%			15.2%			15%			20%		
S_{12}	Q	R	S_{12}	Q	R	S_{12}	Q	R	S_{12}	Q	R	S_{12}	Q	R	S_{12}	Q	R	S_{12}	Q	R
0.241	4.73	1.14	0.475	6.68	3.18	0.540	7.43	4.01	0.599	8.04	4.82	0.895	7.2	6.44	0.533	7.39	3.94	0.701	7.32	5.13

RESULTS AND DISCUSSION

Retention time data were recorded at 170° ($\pm$ 1°), and the retention distances and band widths were measured from charts. These data are presented in Table I. The recorder chart speed was maintained as shown in this table. The last two columns give the data recorded for columns filled with Celite packing material.

With these observations, a direct comparison between the two types of support is rendered possible. For this purpose, values of 'S', 'Q' and 'R' have been calculated for a compound pair, longicyclene and carvone and are presented in Table II[7].

It can be seen clearly that the values obtained for the 5 % and 9 % columns of fire-brick support are comparable to those obtained on the Celite columns (15 % and 20 %, respectively). Similarly "HETP" values* for all the compounds have been calculated and presented in Table III.

TABLE III

EFFECT OF LIQUID LOADING ON "HETP" VALUES (mm)

Compound	Percentage of stationary phase on fire-brick support					Percentage of stationary phase on Celite 545	
	1 %	3 %	5.75 %	9.0 %	15.2 %	15.0 %	20.0 %
Longicyclene	7.27	3.60	2.79	2.52	4.49	2.74	3.28
Longifolene	4.50	3.47	2.60	2.61	3.14	2.17	3.20
Humulene	3.47	3.55	2.14	2.03	2.83	2.25	2.76
Carvone	5.59	2.79	2.26	1.94	2.41	2.30	2.33
Caryophyllene	8.94	5.0	2.64	2.37	5.69	2.84	2.66
Camphor	4.13	3.33	2.48	2.91	3.47	3.00	—
Borneol	3.99	2.62	2.55	2.20	—	2.78	2.56
Dihydro ar-curcumene	12.2	—	2.29	2.53	2.49	3.23	2.66
Ar-himachalene	5.47	—	1.90	1.70	1.93	2.37	2.60

It was noted by several workers that an ideal support should be sufficiently porous and hard and it should not have any catalytic effect. Recently, SAHA AND GIDDINGS, while studying physical aspects and their effect on efficiencies of various Chromosorbs, showed that the comparison of two supports having a different density at equal percentage substrate does not furnish the real picture of the performance[9]. A more realistic comparison is obtained for equal "β" values[10]. This value is obtained by ratio of gas volume to liquid volume of the stationary phase, in a typical GLC column.

For the calculation of such type of "β" values, one should know the density of the stationary phase at the column temperature. For mere simplicity, we have taken this value as "one" and compiled the "β" values for all the columns (Table IV). The assumption of the density will not affect conclusions because the same stationary phase and constant temperature have been used for all the columns. Table IV furnishes a clear comparison between our laboratory made support and "Celite 545".

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* These values seem to be rather high. This may be due to the fact that we have not used injection port heaters and the quantity of the sample was rather high. These parameters have a pronounced effect on HETP[8].

TABLE IV

EFFECT OF LIQUID LOADING ON PRESSURE GRADIENT AND β VALUES

P_i/P_0 values and "β" values.

Percentage of stationary phase on fire-brick support										*Percentage of stationary phase on Celite 545*			
1%		*3%*		*5.75%*		*9.0%*		*15.2%*		*15%*		*20%*	
P_i/P_0	β	P_i/P_0	β	P_i/P_0	β	P_i/P_0	β	P_i/P_0	β	P_i/P_0	β	P_i/P_0	β
1.520	86.7	1.440	31.6	1.450	17.7	1.52	—	1.355	5.9	1.49	16.6	1.237	15.3

It is interesting to find that when the "β" values of "fire-brick" columns coincide with those of the Celite columns the resolution "R" values also coincide. This fact indicates that the efficiency of our laboratory made base is quite comparable with that of well established "Celite" supports.

Next, in order to find out the usefulness and limitations of our fire-brick support, the effect of variation of flow rate was studied. For this purpose, a column having 5.75 % stationary phase was used. Results are recorded in Table V. Samples chosen, were menthyl acetate, n-decanol, carvone and longifolene. A plot of flow rate at the column outlet against the HETP values gives the typical hyperbolic curves as shown in Fig. 1. HETP values are sufficiently low even at high flow rates. This fact in turn has rendered possible speedy analysis without losing efficiency of the column.

TABLE V

"HETP" VALUES AT DIFFERENT FLOW RATES

Temperature: 171°.

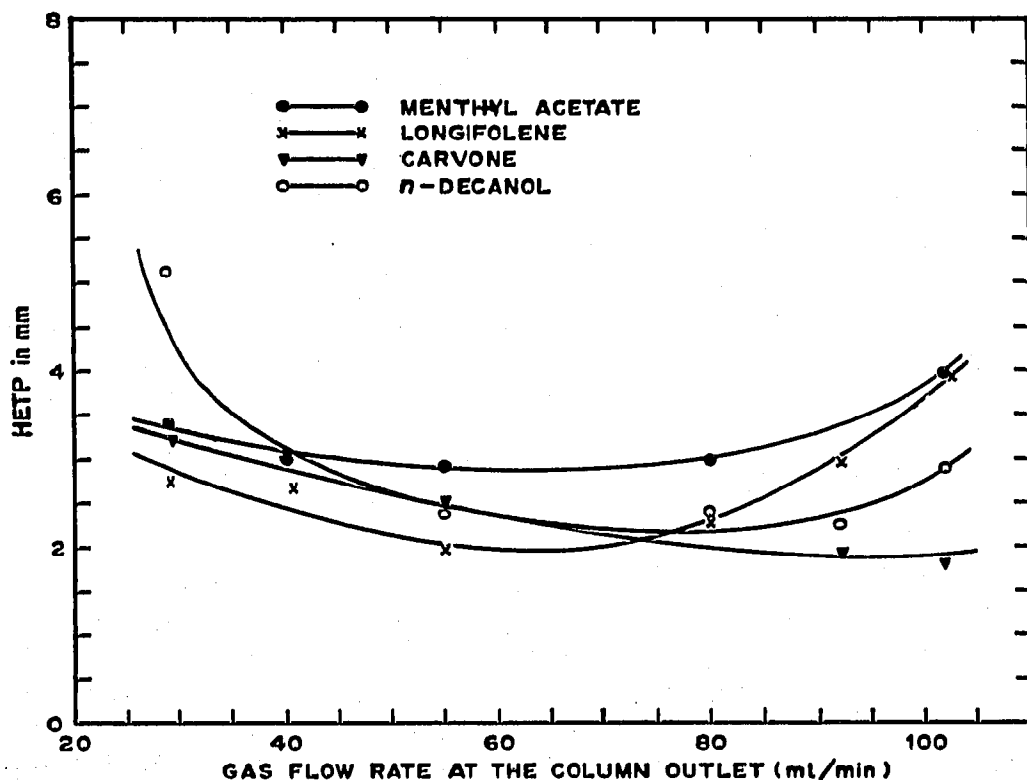| Compound	Carrier gas flow rate at the column outlet					
	102 ml/min	92 ml/min	80 ml/min	55 ml/min	41 ml/min	29 ml/min
Menthyl acetate	3.98	3.81	2.96	2.92	3.01	3.38
Longifolene	3.98	2.99	2.27	1.97	2.65	2.75
Carvone	1.84	1.99	2.37	2.50	—	3.25
n-Decanol	2.86	2.27	2.44	2.45	2.43	5.15

Fig. 1. HETP *versus* gas flow rate, showing effective range of gas flow without appreciable increase in HETP.

Similarly it is worthwhile noting that in a graph of HETP values against the percentage of the stationary phase (Fig. 2), HETP values do not change much, even though the percentage of stationary phase is varied from 4 to 12.5.

These observations indicate that our supporting material is sufficiently porous and can be used efficiently. Thus it can be concluded that a supporting base with somewhat high packing density but having sufficient porosity can be effectively used, if the weight of the stationary phase is properly adjusted.
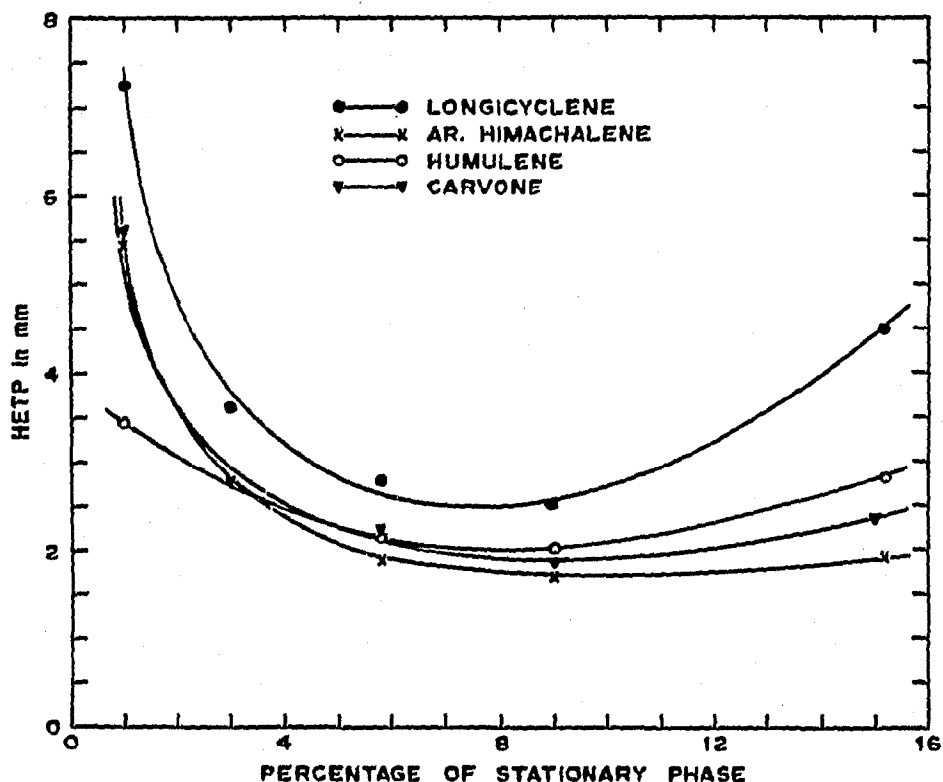
Fig. 2. Effect of liquid loading on HETP values.

The real usefulness of this type of supporting material may be appreciated by looking at Fig. 3. Satisfactory resolution of four monoterpenic alcohols is rendered possible, without decomposition, within barely 10 min.

Analysis of high boiling polar compounds is generally affected by the nature of the supporting material. For such an analysis, a low percentage of the stationary phase is usually required. But this in turn leaves the active sites on the supporting material uncovered.

In order to test this effect, a column was run as follows: It contained only 4% stationary phase; a blend of Carbowax 4000 and succinic acid–diethylene glycol polyester. Analyses of several high boiling terpenic compounds were performed at 220°. Chromatograms of two high-boiling sesquiterpenic lactones, hexahydrocostunolide, b.p. 135–138°/0.4 mm and hexahydrodehydrocostus lactone, b.p. 130–135° per 0.5 mm are given (Figs. 4a and b)[11, 12]. These analyses have revealed that high boiling lactones and carbonyls are satisfactorily resolved without appreciable tailing. However, when high boiling n-alkanols were analysed on this column, peaks were somewhat distorted. This may be due in part to the fact that no "pre-heater" was

used during these analyses and partly due to the somewhat adsorptive nature of the supporting material.

These points are being further investigated after deactivation of the supporting material by silanisation or by PVP treatment[18].
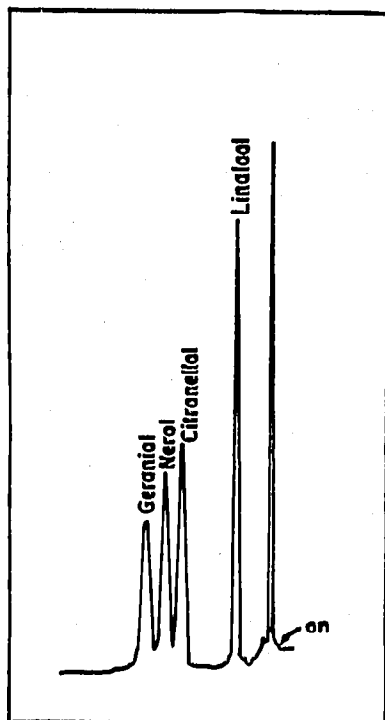

Fig. 3. Chromatography of monoterpenic alcohols. Temperature: 172° ($\pm$ 1°). Carrier gas: hydrogen, 4 l/h. Stationary phase: 4% Carbowax 4000, on 120–150 mesh fire-brick powder.
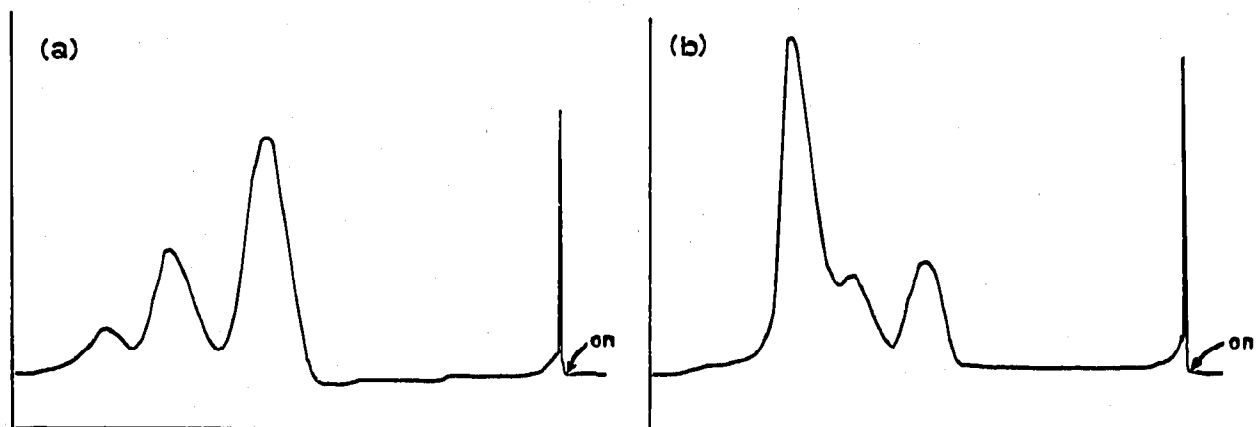

Fig. 4. Chromatography of high-boiling sesquiterpenic lactones, (a) hexahydrocostunolide and (b) hexahydrodehydrocostus lactone. Temperature: 220° ($\pm$ 1°). Carrier gas: hydrogen, 4 l/h. Stationary phase: a blend of succinic acid–diethylene glycol polyester and Carbowax 4000 (4%) on 120–150 mesh fire-brick powder.

SUMMARY

   A useful supporting material for GLC columns has been developed from locally available Indian fire-brick and it was systematically compared with "Celite 545". Calculation of the values of 'S', 'Q', 'R' and "HETP" for both types of columns, has furnished a real comparative study. The concept of "β values" seems to be very useful for such a comparison. After proper adjustment of the weight of the stationary phase, it is possible to construct quite efficient columns. It was observed that this supporting material does not have a catalytic effect. It was possible to analyse mixtures of labile monoterpenic alcohols, without any decomposition.

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