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Characterization and selection of stationary phase for Gas -Liquid Chromatography

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ABSTRACT

The function of the stationary liquid phase is to separate the sample components into discrete peaks. In addition, the liquid phase should have reasonable chemical and thermal stability. In order to function efficiently the stationary phase must remain in the liquid condition. Usually the lower temperature limit operations are kept 10-15 ^{0} C below the upper temperature limit. The amount of column bleed (vaporization of the stationary liquid phase) must be minimized to prolong the column life, to prevent any fouling of the detector, and to maintain baseline stability on the chromatogram. It makes possible the separation of solutes with very similar properties: On the basis of their behavior with a solute, conclusions may be drawn concerning the nature and the general characterization of the particular solute; they contribute useful data to the knowledge of molecular interactions. Currently, no adequate directions are available for selection of columns. However selection has been proposed through matching of polarities of solutes with stationary phases. Certain theories based on Kovats retention indices and McReynolds Theory could be considered for this purpose. Improved resolution between the peaks was the primary objective for the new column selection, which was carried out based on the different models viz., Kovats retention indices and McReynolds Theory.

Key words*:* Kovats retention indices, polarity, stationary phase, McReynolds constants, Gas chromatography.

INTRODUCTION

A nearly unlimited number of liquid stationary phases [1] are available in gas chromatography, if one considers that mixture of stationary liquid can also be used. A classification of stationary phases and a drastic reduction in their number to a standard set are highly desirable in the interest of rationalization. The experimental choice of a single or several stationary phases for the separation of a given mixture by gas-liquid chromatography should be confined to a search in the smallest possible number of solvents. In the identification of solutes by means of multidimensional retention data, only stationary phases that make a significant contribution to the information content of the data should be applied. In order to achieve this objective, the number of liquid stationary phases should be reduced to those solvents which have significant dissimilar retention characteristics [2-5]. The systematic reduction of the number of stationary phases requires their ranking in order of their effect on the retention pattern.

Retention indices are rapidly becoming accepted as the method of choice for reporting chromatographic data.

The retention index (RI) indicates where a compound will appear on a chromatogram with respect to n- paraffins; with the use of relative retention times. There is no restriction on the reference substance for relative retention; consequently hundreds of reference materials have been used as found in the literature.

By definition, the RI for the normal paraffins is equal to 100 times the number of carbon atoms in the compound, regardless of the column used or the chromatographic conditions; the RI for pentane is 500, for n-hexane-600, for n- heptane- 700, etc. To determine the retention index for benzene on a squalane column, benzene and any two other n- paraffins, are chosen such that one elutes before benzene and other afterwards eg. Benzene elutes approximately midway between n-hexane (600) and n-heptane (700). To support the rule, n-hexane has been found to elute before benzene in the chromatogram and n-heptane after elution of benzene. A plot of the logarithm of the **adjusted retention time** (t'_R) verses the retention indices gives a straight line for n-paraffins. From this plot it is possible to determine the retention indices of other compounds by determining the value corresponding to their log $t'_{R.}$

The RI values for n-paraffins are definitions and apply regardless of the conditions. RI values for other compounds cannot be true unless complete chromatographic conditions are provided.
For example, benzene has RI value of 649 on a column consisting of 20% squalane on Chromsorb W, with column operated at 100^0 C, while on polyethylene glycol, G-20 molecular weight 380-420 (20% Repolex 400 column) on Chromsorb W at 100^0 C, the RI value was found to be 1005. Hence RI value is considerably greater for benzene on 20% Repolex column. The difference in the RI can be expressed as

$\Delta I = I_{polar} - I_{non\ polar} = ax$. This difference in the indices (ax) is proportional to the column polarity "X". It is possible to characterize a column by comparison of the retention index of a compound analysed on a polar phase to the retention index of the same compound on the non polar phase.

Materials and Methods:

Solvents were selected based on industrial survey, reference standard i.e. methanol, ethanol, acetone, isopropyl alcohol, dichloromethane, acetonitrile, hexane, ethyl acetate, xylene, tetrahydrofuran, heptane, 1-4-dioxane, toluene, pyridine, butyl acetate and N, N –dimethyl formamide were purchase from Ranbaxy, Mumbai, Rankem, Mumbai , and Thomas Baker, Mumbai. Intermediate polar column **BP 624 column** (6 % Cyano propyl phenyl and 94 %

dimethyl polysiloxane, 30m X 0.53mm i.d. X 0.25µm coating thickness) from SGE (Australia) and Non polar column **EC-5 column** (5 % phenyl and 95 % dimethyl polysiloxane, 30m X 0.53mm i.d. X 0.25µm coating thickness) purchased from Alltech.

RESULTS AND DISCUSSION

Kovats retention index [6-10] for various solutes on different stationary phases are calculated by following group increment contribution method. To calculation of Kovats retention indices for methanol on non polar stationary phase [6]. Increment of 112 for CH_3 and 255 for OH, giving a net increment of 367, which is considered as KRI for methanol on non polar stationary phase. Calculation of Kovats retention indices for methanol using group increments contributions for intermediate polar stationary phase (IPSP). Increment of 112 for CH_3 and 255 for OH + 11 (Increment for IPSP), giving a net increment of 378, which is considered as KRI for methanol on intermediate polar stationary phase. To calculation Kovats retention indices for methanol on polar stationary phase. Increment of 113 for CH_3 and 747 for OH, giving a net increment of 860, which is considered as KRI for methanol on polar stationary phase. Similarly the KRI for various solutes have been calculated on non polar, intermediate polar and polar column and the same is presented in Table 1, 2 and 3 respectively.

Table 1: Calculation of Kovats retention indices (KRI) for various solutes on Non Polar Column [6]

Sl .No	Name	Structural Formula	Group Increments	Increment Value	Kovats Retention Index
1	Methanol	CH_3OH	CH_3 = 112 OH = 255	1x112 = 112 1x 255 = 255	367
2	Ethanol	CH_3CH_2OH	CH_3 = 112 CH_2 = 99 1^0 OH = 255	1x112 = 112 1x 99 = 99 1x 255 = 255	466
3	Acetone	CH_3 CO CH_3	CH_3 = 112 CO = 235	2x 112 = 224 1x 235 = 235	459
4	Iso propyl alcohol	$(CH_3)_2$ CHOH	CH_3 = 112 CH = 22 2^0 OH = 189	2x 112 = 224 1x 22 = 22 1x 189 = 189	435
5	Dichloromethane	CH_2Cl_2	CH_2 = 99 Cl_2 = 217	1x 99 = 99 1 x 217 =217	415
6	Acetonitrile	CH_3 CN	CH_3 = 112 CN= 354	1x 112= 112 1x 354 = 354	466
7	n- Hexane	C_6H_{12}	CH_2 = 99 CH_3 = 112	4x 99 = 396 2x112 = 224	620

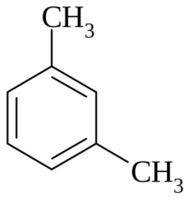
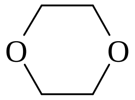
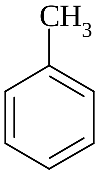
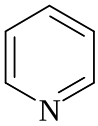
8	Ethyl acetate	$CH_3\ COOCH_2CH_3$	CH_3 = 112 CH_2 = 99 COO = 266	2x 112 = 224 1x 99 = 99 1x 266 = 266	589
9	Tetrahydrofuran	$(CH_2)_4O$	r- CH_2 = 121 r- O = 112	4x 121 = 484 1x 112 = 112	596
10	Xylene		CH_3 = 112 =CH = 110 =C = 90	2x 112 = 224 4 x 110 = 440 2 x 90 = 180	844
11	n- Heptane	$CH_3\ (CH_2)_5\ CH_3$	CH_3 = 112 CH_2 = 99	2 x 112 = 224 5 x 99 = 495	719
12	1-4- dioxane		r- CH_2 = 121 r- O = 112	4 x 121 = 484 2 x 112 = 224	708
13	Toluene		r –CH = 121 CH_3 = 112	5 x 121=605 1 x 112 = 112	717
14	Pyridine		r-CH = 110 r- N = 116	5 x 110 = 550 1 x 116 = 116	666
15	N-N- Dimethyl formamide	$HCON(CH_3)_2$	CH_3 = 112 HCO = 299 N = 38	2 x 112 = 224 1 x 299 = 299 1 x 38 = 38	561
16	Butyl acetate	$CH_3COO(CH_2)_3\ CH_3$	CH_3 = 112 CH_2 = 99 COO = 266	2 x 112 = 224 3 x 99 = 297 1 x 266 = 266	787

Table 2: Calculation of Kovats retention indices (KRI) for various solutes on Intermediate Polar Column [6]

Sl.No	Name	Structural Formula	Group Increments	Increment Value	Kovats Retention Index
1	Methanol	CH_3OH	CH_3 = 112 OH = 255	1x112 = 112 1x 255 = 255	378

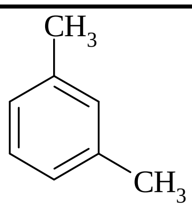
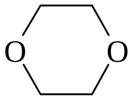
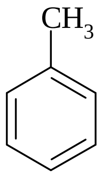
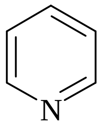
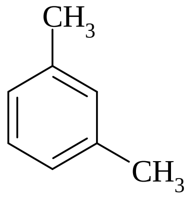
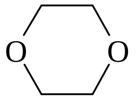
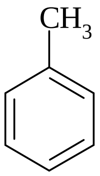
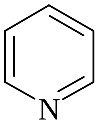
2	Ethanol	CH_3CH_2OH	CH_3 = 112 CH_2 = 99 1^0 OH = 255	1x112 = 112 1x 99 = 99 1x 255 = 255	477
3	Acetone	CH_3 CO CH_3	CH_3 = 112 CO = 235	2x 112 = 224 1x 235 = 235	470
4	Iso propyl alcohol	$(CH_3)_2$ CHOH	CH_3 = 112 CH = 22 2^0 OH = 189	2x 112 = 224 1x 22 = 22 1x 189 = 189	446
5	Dichloromethane	CH_2Cl_2	CH_2 = 99 Cl_2 = 217	1x 99 = 99 1 x 217 =217	426
6	Acetonitrile	CH_3 CN	CH_3 = 112 CN= 354	1x 112= 112 1x 354 = 354	477
7	n- Hexane	C_6H_{12}	CH_2 = 99 CH_3 = 112	4x 99 = 396 2x112 = 224	631
8	Ethyl acetate	CH_3 $COOCH_2CH_3$	CH_3 = 112 CH_2 = 99 COO = 266	2x 112 = 224 1x 99 = 99 1x 266 = 266	600
9	Tetrahydrofuran	$(CH_2)_4$ O	r- CH_2 = 121 r- O = 112	4x 112 = 484 1x 112 = 112	607
10	Xylene		CH_3 = 112 =CH = 110 =C = 90	2x 112 = 224 4 x 110 = 440 2 x 90 = 180	855
11	n- Heptane	CH_3 $(CH_2)_5$ CH_3	CH_3 = 112 CH_2 = 99	2 x 112 = 224 5 x 99 = 495	730
12	1-4- dioxane		r- CH_2 = 121 r- O = 112	4 x 121 = 484 2 x 112 = 224	719
13	Toluene		r –CH = 121 CH_3 = 112	5 x 121=605 1 x 112 = 112	728
14	Pyridine		r-CH = 110 r- N = 116	5 x 110 = 550 1 x 116 = 116	677
15	N-N-Dimethyl formamide	$HCON(CH_3)_2$	CH_3 = 112 HCO = 299 N = 38	2 x 112 = 224 1 x 299 = 299 1 x 38 = 38	572
16	Butyl acetate	$CH_3COO(CH_2)_3$ CH_3	CH_3 = 112 CH2 = 99 COO = 266	2 x 112 = 224 3 x 99 = 297 1 x 266 = 266	798

Table 3: Calculation of Kovats retention indices (KRI) for various solutes on Polar Column [6]

Sl.No	Name	Structural Formula	Group Increments	Increment Value	Kovats Retention Index
1	Methanol	CH_3OH	$CH_3 = 113$ $OH = 747$	1x113 = 113 1x 1747	860
2	Ethanol	$CH\ _3CH_2OH$	$CH_3 = 113$ $CH_2 = 99$ 1^0 OH = 397	1x112 = 113 1x 99 = 99 1x 397 = 397	609
3	Acetone	CH_3 CO CH_3	$CH_3 = 113$ CO = 524	2x 112 = 226 1x 524 = 524	750
4	Iso propyl alcohol	$(CH_3)_2$ CHOH	$CH_3 = 113$ CH = 06 2^0 OH = 561	2x 112 = 226 1x 06 = 06 1x 561 = 561	793
5	Dichloromethane	CH_2Cl_2	$CH_2 = 99$ $Cl_2 = 396$	1x 99 = 99 1 x 396= 396	495
6	Acetonitrile	CH_3 CN	$CH_3 = 113$ CN= 781	1x 112= 113 1x 354 = 781	894
7	n- Hexane	C_6H_{12}	$CH_2 = 113$ $CH_2 = 99$	2x 113= 226 4x 99 = 396	622
8	Ethyl acetate	CH_3 $COOCH_2CH_3$	$CH_3 = 113$ $CH_2 = 99$ COO = 515	2x 113 = 226 1x 99 = 99 1x 515 = 515	840
9	Tetrahydrofuran	$(CH_2)_4$ O	r- $CH_2 = 128$ r- O = 202	4x 128 = 512 1x 202 = 202	714
10	Xylene		$CH_3 = 113$ =CH = 159 =C = 122	2x 113 = 226 4 x 159 = 636 2 x 122 = 244	1106
11	n- Heptane	$CH_3\ (CH_2)_5\ CH_3$	$CH_3 = 113$ $CH_2 = 99$	2 x 113 = 226 5 x 99 = 495	721
12	1-4- dioxane		r- $CH_2 = 128$ r- O =202	4 x 128= 512 2 x 202 = 404	916
13	Toluene		r –CH = 159 $CH_3 = 113$ = C- = 122	5 x 159 = 795 1 x 113=113 1 x 122= 122	1030
14	Pyridine		r-CH = 159 r- N = 227	5 x159= 795 1 x 227= 227	1022
15	N-N- Dimethyl	$HCON(CH_3)_2$	$CH_3 = 113$ HCO = 602	2 x 113 = 226 1 x 602 = 602	956

	formamide		N = 128	1 x 128 = 128	
16	Butyl acetate	$CH_3COO(CH_2)_3\ CH_3$	CH_3 = 113 CH_2 = 99 COO = 515	2 x 113= 126 3 x 99 = 297 1 x 5154= 515	1038

McReynolds Theory:
On the basis of "nearest neighbour technique [11-14]" column selection can be carried out with McRenyolds constants ΔI values for the solutes determined on 226 stationary phases, a classification to determine groups and similarities of phases has been established. A series of twelve generally acceptable phases covering the entire range of polarities as shown by their nearest neighbour distances from squalane has been proposed on the basis of the distances of other phases from the nearest appropriate preferred phases. The nearest neighbour technique was chosen by Huber JFK [1] for its ability to point out true similarity in high dimensional data. In this technique, the data are represented as n-dimensional pattern vectors. Patterns that exist close together in the n- dimensional space are considered to be similar, with the degree of similarity determined by Euclidian distance between patterns. When comparing phase, the distance is an effective guide as to whether one particular phase can be replaced by another.
The distance (D) between phases A and B is calculated by

$$D = [\sum_{i=1}^{m} (\Delta I_{Ai\,-}\ \Delta I_{Bi})^2\ ]^{1/2}$$

Where, i represent the compound run on both phases. The result of this calculation is that for each liquid phase, the most similar liquid phase has the smallest value of D, and the least similar, the largest. The calculations were performed using the ΔI values provided by five dimensions used in McRenyolds summation. The five standard compounds were considered to be sufficient for the characterization of most liquid phases. While the interest in the neighbour technique has been used for the selection of groups and similarity of stationary phases. By determining the nearest neighbour distances on a series of phases for particular homologous compounds, the distances or differences from dimethyl polysiloxane as the low polarity base were obtained and simple comparison allows selectivity to be observed more readily than from a consideration of McReynolds constants where frequently an increase in one of the values is accompanied by increase in the other terms. It is important to classify each of the stationary phase by its ability to retard specific functional groups. Stationary phase can be classified by the method developed by McReynolds. It involves measurement of the Retention Index (RI). This is a measure of the degree to which polar compounds are retarded relative to their elution on a non-polar phase. A systematic method for expressing retention data uses Kovats retention index (RI). These indices indicate where compounds will appear on a chromatogram with respect to straight chain alkanes injected with the samples, for selected "index" compounds on a column packed with a particular stationary phase. These RI values are compared with the RI values for the same index compounds obtained on a squalane column. The difference determines the degree to which each index compound is retarded by the stationary phase. It is a measure of solute- solvent interaction due to all intermolecular forces other than London dispersion forces. The latter are the principal solute- solvents effects with squalane.

The overall effects due to hydrogen bonding, dipole moment, acid- base properties, and molecular configuration can be expressed as

$$\sum \Delta I = ax' + by' + cz' + du' + es'$$

In this expression,
x' = ΔI for benzene (Intermolecular forces typical of aromatic and olefin compounds)
y' = ΔI for 1- butanol (an electron attractor typical of alcohols, nitriles, acids, nitro compounds, and alkyl mono, di- , and trichlorides)
z' = ΔI for 2- pentanone (an electron repeller typical of ketones, ethers, aldehydes, esters, epoxides, and dimethyl amino derivatives)
u' = ΔI for 1- nitro propane (typical of nitro and nitrile compounds)
s' = Δ I for pyridine.

D-values (Nearest neighboring distance) for a series of stationary liquid phases have been calculated based of McReynolds constant values for solutes on various stationary phases. The polarity of one standard stationary phase is kept constant while the polarity behavior of other stationary phase is varied. The method adopted for calculating D-value of Poly dimethyl siloxane (G 36) with respect to the standard considered for the study Poly diphenylvinyl dimethylsiloxane (OV-101) is given below the same is presented in Table 4.

$$\begin{aligned}
D &= [\sum_{i=1}^{m} (\Delta Ai - \Delta Bi)\, 2]^{1/2} \\
&= [\sum_{i=1}^{m} (\text{Difference})\, 2]^{1/2} \\
&= [\sum_{i=1}^{m} (17-33)\, 2 + (57-72)2 + (45-66)2 + (67-99)2 + (43-67)2]^{1/2} \\
&= [\sum_{i=1}^{m} (-16)\, 2 + (-15)2 + (-21)2 + (-32)2 + (-24)2]^{1/2} \\
&= [\sum_{i=1}^{m} (256 + 225 + 441 + 1024 + 576)]\, ½ \\
&= (2513)^{1/2} \\
&= 50.12
\end{aligned}$$

D-value for G-36 with respect to OV-101 is 50.12; Similarly D values for other set of stationary phases were calculated as presented in Table.4

Table.4 The relation ship between general polarities vs. McReynolds five constants were plotted

Sl.No	Column	GP	D Values
0	Poly dimethylsiloxane(OV-101)	229	----------
1	Poly diphenylvinyl dimethylsiloxane (G 36)	337	50.12
2	6% cyanopropyl phenyl 94% dimethyl polysiloxane(G43)	423	89.9

3	Polycarborane methyl Siloxane (G33)	474	119
4	5% Phenyl-95% methylpolysiloxane (G27)	587	164.79
5	Polydiphenyldimethylsiloxane 20:80 (G32)	592	168.03
6	Polydiphenyldimethylsiloxane 50:50 (G3)	884	300.19
7	Polycarboranemethylcyanoethyl silicone (G 41)	952	348.73
8	Polytrifluoropropoyl methylsiloxane (G6)	1545	628.44
9	Polycyanopropoyl methylsiloxane (G 19)	1813	725.25
10	Polyethylene glycol (G16)	2424	898.39
11	Carbowax (G7)	3200	1353.04
12	Polyethylene glycol adipate (G23)	3682	1569.46

For the selection of column, the separation patterns of solutes over columns of different polarity have been studied. The relationship of **KRI for solutes** over **stationary phases of different polarities** (calculated as General Polarity (GP) using nearest neighboring technique) has been graphically presented in Fig 1.

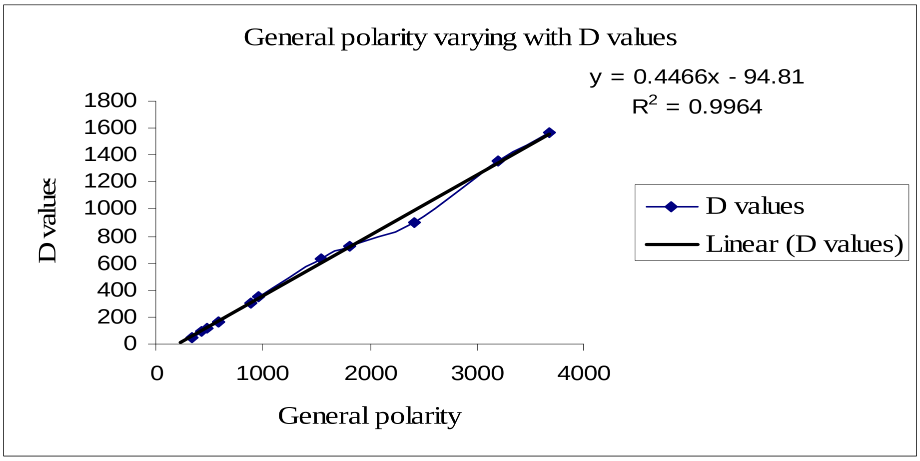


Fig.1 General Polarity Vs D values

Solutes were classified as x', y', z' and s' based upon their properties as per McReynolds constants and presented in Table.5.

Table. 5 Classification of solutes into x', y', z' and s'

Sl. No	solutes	Kovats RI NP	Kovats RI IP	Kovats RI Polar
1	n- Hexane (x')	620	631	622
2	Xylene (x')	844	855	1106
3	n- Heptane (x')	719	730	721
4	Toluene (x')	717	728	1030
5	Methanol (y')	367	378	860
6	Ethanol (y')	466	477	959
7	IPA (y')	435	446	793
8	DCM (y')	415	426	495
9	ACN (y')	466	477	894
10	Ethyl acetate (z')	589	600	840
11	Tetrahydrofuran (z')	596	607	714
12	DMF (z')	561	572	956
13	Butyl acetate (z')	787	798	1038
14	Acetone (z')	459	470	750
15	1-4- dioxane (z')	708	719	916
16	Pyridine (s')	666	677	1022

Peak positions for x' like n-hexane, n-heptane, toluene and xylene type solutes are moderately resolved over GC columns of varying polarity as justified through a plot of their KRI over columns of different polarities. Fig.2 shows a plot with high slope y', s' and z' type of solutes distribute and get well resolved over GC columns. As KRI of x', y', s' and z' solutes are calculated based on KRI to longer, the Retention time for such solutes is expected to be longer. Hence polar column were not prefred for the study.

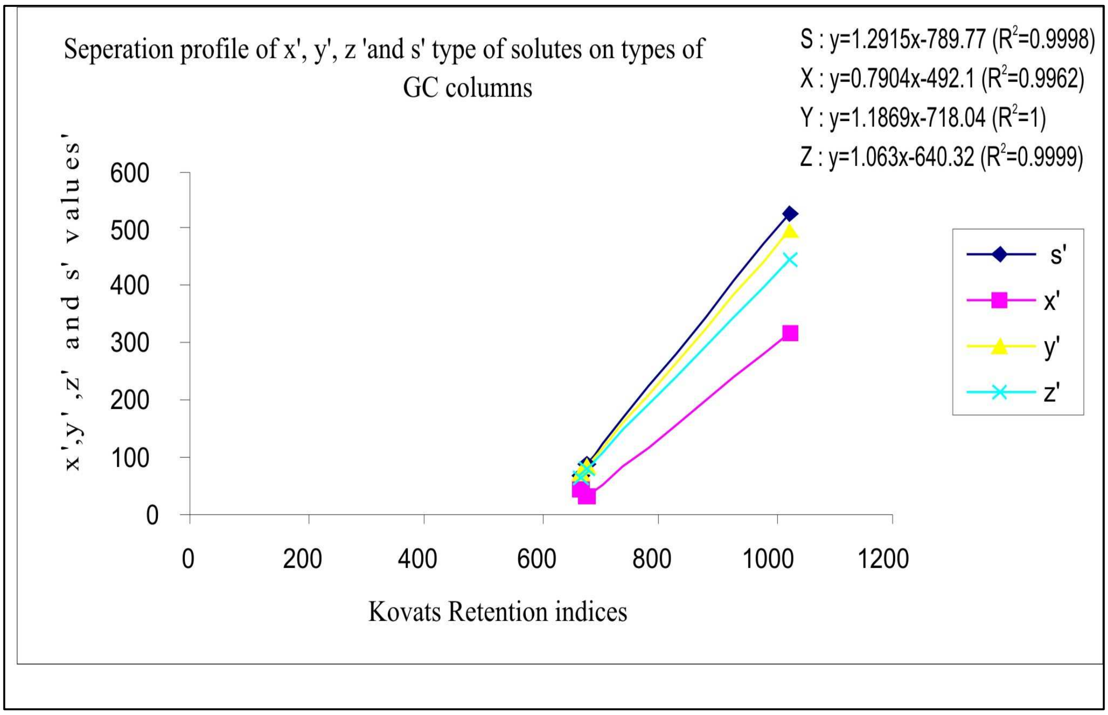


Fig.2 Separation profile x', y', z' and s' type of solutes vs type of GC columns

Observations concluded form the graphs, for non polar solutes the polarity factor x' varies proportionately with KRI, the polarity factor varying between small units of 285 units over KRI of about 200. Similarly for y' the polarity factor varies 400 units, which varies in KRI about 500 units, for z' the polarity factor varies 360 units which varies in KRI about 250 units, for s' the polarity factor varies 450 units which varies in KRI of about 250 units. Hence for moderately and polar solvents the intermediate and non polar columns provide well distributed retention indices.

For polar columns the polarity factors of solutes comparatively provide very high KRI, it is proposed that retention time may be unduly high on polar columns, for majority of the solutes. Hence it is proposed to use moderately polar and non polar columns for the proposed study.
Slope of linear regression lines for analysis of solutes by proposed method over columns varying in polarity were found as follows methanol as 5.643, ethanol as 5.534 , acetone as 9.631, isopropyl alcohol as 7.803, dichloromethane as 36.41, acetonitrile as 6.511, hexane as -89.41, ethyl acetate as 11.198, xylene as 10.719, tetrahydrofuran as 24.31, heptane as -89.41, 1-4-

dioxane as 13.56 , toluene as 8.943, pyridine as 7.847, butyl acetate as 11.17 and N, N – dimethyl formamide as 7.062.

CONCLUSIONS

It was found that a specific pattern of separation over columns ranging in polarity from non-polar to polar was observed for solutes like methanol, ethanol, acetone, isopropyl alcohol, acetonitrile, ethyl acetate, xylene, dioxane, toluene, pyridine, N,N-dimethyl formamide and butyl acetate where slopes varying from 5- 15 was observed as shown in

However for solutes like dichloromethane, tetrahydrofuran, the slope was very high and ranged from 25- 40. For n-hexane and n- heptane, being non polar solutes (hydrocarbon) negative slope was observed. As the solutes selected for study were moderately polar or polar, the variation in slope patterns for hydrocarbons as observed above did not interfere in our hypothesis.

A predictable pattern of separation was observed when moderately polar or non polar columns were used for separation. In case of polar columns, there was no proportional increase in KRI with increase in polarity of stationary phases; hence polar columns were not preferred for the study as satisfactory separation for hydrocarbons could not be achived. In such cases it is likely that the non polar solutes are likely to merge together with no separation on polar columns. The predictable pattern of separation for solutes on moderately polar and non polar columns enabled selection of BP-624 and EC-5 for the proposed study. Intermediate polar column **BP 624 column** (6 % Cyano propyl phenyl and 94 % dimethyl polysiloxane, 30m X 0.53mm i.d. X 0.25μm coating thickness) and Non polar column **EC-5 column** (5 % phenyl and 95 % dimethyl polysiloxane, 30m X 0.53mm i.d. X 0.25μm coating thickness). Non polar column, were selected for the proposed study.

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