Research Article

Polyoxyethylenated Cholesterol – Stationary Phases For Gas Chromatographic Packed Columns

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ABSTRACT

Polyoxyethylenated cholesterol nonionic surfactants with different polyoxyethylene (POE) chain lengths, were employed as stationary phases for gas-liquid partition chromatographic packed columns. Resolution efficiency of these columns were compared with that of polyethylene glycols (PEGs) known as carbowaxes having comparable polyoxyethlene (POE) chain lengths. Polarity is compared through resolution measurements of polar and nonpolar solute pair on two packed columns of equal loading and dimension; one contains 10% of the prepared nonionic and the other 10% of carbowax. The study revealed that polyoyethylenated cholesterol stationary phases are less polar than carbowaxes. The reduced polarity of nonionic is attributed to the multiring structure of the cholesteryl tail. The recorded chromatograms show that 10% stationary phase in each of the prepared nonionic packed columns, are well suited to base line separation of mixtures of organic compounds including cis/trans isomers through sharp symmetrical peaks, satisfactory resolution in a short retention time.

Keywords: GC phases, Carbowax, Polyoxyethylenated Cholesterol, Nonionic Surfactants, Packed Columns

INTRODUCTION

Polyethylene glycols (PEGs) are the most commonly used materials as stationary phases, sold under the trade name of carbowax having the general structure HO $(CH_2CH_2O)_nH$. The variation in the number (n) of oxyethlene units gives rise to the various carbowax types such as: carbowax 200, carbowax 400, and carbowax 600. These materials provide excellent general polar stationary phases which are used specifically in packed columns for the separation of mixtures of alcohols, esters, aldehydes and ketones[1-4]. Another group of materials is the polyoxyethylene (POE) nonionic surfactants which have the general structure HO $(CH_2CH_2O)R$, where R is the hydrophobic tail group which is adducted to an (n) number of oxyethlene (OE) units representing the polar head-group[5-7]. R, in most nonionics, is either straight-or branched-chain structure[8,9]. More bulky hydrophobic tails are found in polyoxyethylenated alkyl- and dialkyl- phenols of (Igepal series)[10,11] and in polyoxyethylenated sorbitan monolaurate, sorbitan monopalmitate and sorbitan monooleate of (Tween series)[12].

Polarity measurements have been carried out through inverse – phase gas chromatography (IGC) using four commercial POE nonionics from the alkylphenol adducts as stationary phases in packed columns of equal dimensions[13-15]. Each of the employed POE octyl-, nonyl-, dodecyl- and dinonylphenol, was adducted with 4 mol of ethylene oxide. The order of decreasing polarity of these nonionics was: octylphenol adduct > nonylphenol adduct > dodecylphenol adduct > dinonylphenol adduct[16]. Values of two other polarity terms namely, apparent methanol carbon number (C_{MeOH}) and polarity index (IP), were measured on polyoxyethyenated C_{10}-C_{18} fatty amines as stationary phases in packed columns[15,17]. The introduction of simulated hydrophobic tail (SHT) and head group weight fraction (F_{HG}) permits the distinction between the polarities of oxyethylene units adducted to any hydrophobic structure[17,18].

In the present work, the resolution efficiency of six chromatographic columns packed with 10% by weight of the prepared polyoxethylenated cholesterol stationary phases with different polyoxyethylene (POE) chain lengths, were compared with another six chromatographic columns of equal dimensions packed with 10% by weight of the well – known carbowax (polyethylene glycol) stationary phases having comparable POE chain lengths.

Also, the efficiency of polyoxyethylenated cholesterol stationary phase in the separation of mixtures of different organic compounds, was attempted.

MATERIALS AND METHODS

Materials

Ethylene oxide cylinder with recommended valve, from Fluka. Sodium methoxide, unhydrous powder catalyst, from Aldrich. Cholesterol, from Prolabo - code No. 22749.18. Ethylene glycols having average molecular weights: 200, 300, 400, 1000 and 1500 from Aldrich.

Gas Chromatograph

Perkin – Elmer Sigma – 3B, equipped with flame ionization detector (FID). Stainless steel (ss) 1/8" i.d. columns. Chromosorb P-AW-DMCS (acid washed – dimethyl – dichlorosilane treated), 100/120 mesh size

inert solid support from Supelco Inc., for GC packed columns.

The Prepared Stationary Phases

Oxyethylenated Cholesterol nonionic surfactants having different oxyethylene (OE) contents, were prepared by the reaction of gaseous ethylene oxide with cholesterol (Figure 1) using batch laboratory-scale unit similar to that reported in our previous work.[19,20] Oxyethylenation reaction was carried out at 160-150 °C and gaseous ethylene oxide pressure of 2.5-2.9 psi using sodium methoxid catalyst. The prepared nonionics were purified[21] and characterized through spectroscopic (^{1}H NMR and FTIR) and thermal (DTA and TGA) analyses[17,18,22].

The Employed Packed Columns

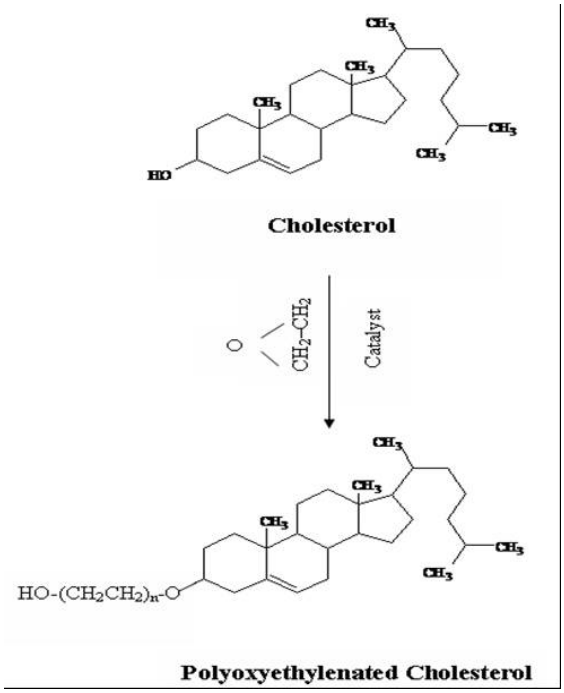

Figure 1: Oxyethylenation Reaction of Cholesterol

Two sets of columns were employed for the sake of comparison. The first set consists of six stainless (ss) columns packed with 10% by wt of the prepared oxyethylenated cholesterol having 4.08, 7.37, 8.91, 13.87, 23.19 and 33.90 OE units, on Chromosorb P-AW-DMCS. In the second set, six ss columns packed with carbowaxes (polyethylene glycols) 200, 300, 400, 600, 1000 and 1500, Having an average number (n) of oxyethylene (OE) units : 4.54, 6.81, 9.08, 13.62, 22.70 and 34.05, respectively. Designations of the employed stationary phases along with the average number (n) of OE units are listed in Tables 1 and 2. The prepared sets of packed columns are listed in Table 3.

RESULTS AND DISCUSSION

Performance of the Prepared Packed Columns

The prepared packed columns are listed in Table 3. After the selection of the proper operating conditions (column temperature and flow rate of mobile phase), three main parameters dictate the performance of any gas-liquid chromatographic (GLC) column: efficiency, resolution and length. Efficiency and resolution can be determined directly from the chromatogram.

Efficiency is a measure of the ability of the column to produce narrow peaks. Efficiency in plates is an easily measured quantity which does characterize the column in terms of peak width[23].

The degree of separation of two solutes is expressed by the resolution (R) which is defined as:

$$R = 2\left(\frac{t_{con}(2) - t_{con}(1)}{W_1 + W_2}\right) \quad \text{.....(1)}$$

$$R = 2\,\frac{\Delta t}{(W_1 + W_2)} \quad \text{.....(2)}$$

Where Δt is the difference between the corrected retention times of the solutes, W_1 and W_2 are the widths of the peaks (the intersection of the tangents to the inflection points with the base line), t_0 is the time required for air peak (a non-retained component).

Table 1: The Prepared Polyoxyethylenated Cholesterol Stationary Phases For GLPC Applications.

St. Phase Designation	Number of oxyethlene units (n)	Average Mol. Wt*	m.p. °C	c.p. °C	Appearance
Chol-4.08	4.08	566	67	40	Soft waxy-like, amber yellow
Chol-7.37	7.37	711	63	42	Soft waxy-like, amber yellow
Chol-8.91	8.91	779	60	69	Soft waxy-like, brown in colour
Chol-13.87	13.87	998	54	88	Soft waxy-like, brown in colour
Chol-23.19	23.19	1408	49	>100	Highly viscous, brown colour
Chol-33.90	33.90	1880	50	>100	Highly viscous, dark brown

GLPC = gas – liquid partition chromatography; n = is the average number of oxyethylene (OE) units / cholesterol molecule.

* C.P = cloud point of 1 wt % aq. solution.; * Av. Mol, Wt = F.W. of cholesterol (386.65) + n (44.05).

Table 2: Polyethylene Glycols (PEG) Used as Stationary Phases in GLPC. H $(OCH_2CH_2)_n$ OH

Designation	n\	Average Mol. Wt	Properties*
PEG200	4.54	200	Liquid, viscosity (210°F) 4.3 centistokes, density 1.127 g/cm^3, m.p. -60°C, n^{20}_D 1.459
PEG300	6.81	300	Liquid, viscosity (210°F) 5.8 centistokes, density 1.125 g/cm^3, m.p. -10°C, n^{20}_D 1.483
PEG400	9.08	400	Liquid, viscosity (210°F) 7.3 centistokes, density 1.128 g/cm^3, m.p. -6°C, n^{20}_D 1.465
PEG600	13.62	600	Liquid, viscosity (210°F) 10.5 centistokes, density 1.128 g/cm^3, m.p. -22°C, n^{20}_D 1.467
PEG1000	22.70	1000	Fused mass, viscosity (210°F) 17.4 centistokes, density 1.101 g/cm^3
PEG1500	34.05	1500	Fused mass, viscosity (210°F) 28 centistokes, density 1.091 g/cm^3

* From Aldrich Catalog Handbook of Fine Chemicals; n\ = The average mol. Wt. of Polyethylene Glycols (PEG) / 44.05

Table 3: Two Prepared Sets of Packed Columns

1-	10% Chol-4.08 on 100/120 mesh chromosorb P AW DMCS, 20 ft × 1/8" SS Column.
2-	10% Chol-7.37 on 100/120 mesh chromosorb P AW DMCS, 20 ft × 1/8" SS Column.
3-	10% Chol-8.91 on 100/120 mesh chromosorb P AW DMCS, 20 ft × 1/8" SS Column.
4-	10% Chol-13.87 on 100/120 mesh chromosorb P AW DMCS, 20 ft × 1/8" SS Column.
5-	10% Chol-23.19 on 100/120 mesh chromosorb P AW DMCS, 20 ft × 1/8" SS Column.
6-	10% Chol-33.90 on 100/120 mesh chromosorb P AW DMCS, 20 ft × 1/8" SS Column.
7-	10% PEG-200 on 100/120 mesh chromosorb P AW DMCS, 20 ft × 1/8" SS Column.
8-	10% PEG-300 on 100/120 mesh chromosorb P AW DMCS, 20 ft × 1/8" SS Column.
9-	10% PEG-400 on 100/120 mesh chromosorb P AW DMCS, 20 ft × 1/8" SS Column.
10-	10% PEG-600 on 100/120 mesh chromosorb P AW DMCS, 20 ft × 1/8" SS Column.
11-	10% PEG-1000 on 100/120 mesh chromosorb P AW DMCS, 20 ft × 1/8" SS Column.
12-	10% PEG-1500 on 100/120 mesh chromosorb P AW DMCS, 20 ft × 1/8" SS Column.

AW DMCS = acid washed – dimethyl dichlorosilane treated; SS = stainless steel.

Stationary Phase Selection

There are two general types of liquid stationary phases: non-polar and polar. The basic principle in selecting a stationary phase is the same as the freshman chemist's rule for selecting a solvent, "like attracts like". A more elegant way of saying the same thing is that chemicals (sample components) dissolve best (are retained longer) by solvents (liquid stationary phases) that are chemically similar to themselves. A useful way of putting this "congeniality" principle to work is to consider liquid phase polarity[23].

Non-polar stationary phase (e.g. squalane, silicone) separates components essentially by boiling point. A series of hydrocarbons will separate with the lowest boiling member appearing first, and a series of alcohols will do the same, but it is not generally possible to say in advance how the two series will overlap in a sample containing both. Even if we establish a rule for the hydrocarbon-alcohol mixture, how can we deal with mixtures containing ethers, esters, aldehydes, ketones or other structures, such a rule will break down.

Polar stationary phase (e.g. carbowax) separates components essentially by polarity difference, the less polar component appears first and the more polar component will be retained on polar stationary phase.

For better illustration between the performance of non-polar and polar column, consider a mixture of cyclohexane and benzene, the boiling points are less than one degree apart so the boiling point column will not do the job. There is a slight polarity difference, but these compounds are both hydrocarbons and have no great affinity for the usual polar stationary phase and column efficiency would be poor. There are two stationary phases which will make the separation based on chemical similarity: Squalane, a saturated hydrocarbon stationary phase, will retain cyclohexane in preference to aromatic benzene; whereas, polyphenyl ethers do just the opposite and cyclohexane appears first. Thus, chemical similarity is a rather nebulous concept, but it can lead to separation which is otherwise extremely difficult. It can be used effectively by chromatographers having a fair amount of chemical experience.

In this application study, the prepared packed columns listed in Table 3, have been tested specifically for the separation of a solute pair which consists of n-heptane, a nonpolar solute b.p.98.4 °C, and 1-propanol, a polar solute b.p.97 °C[24,25]. The solute pair is injected on two stainless steel columns of equal dimensions and under the same operating conditions, the first column is packed with 10% polyoxyethylenated cholesterol with a certain number (n) of OE units, and the second is packed with 10% polyethylene glycol (carbowax) which has almost the same number of OE units. For example, the performance

of 10% Chol-7.37 is compared with 10% PEG-300 and the performance of 10% Chol-33.90 is compared with 10% PEG-1500 and so on (n values of the examined stationary phases are listed in Tables 1 and 2). This variety of column packings exhibit the efficiency to resolve the injected solute pair. Comparison of the obtained chromatograms shows the increased resolving power of carbowax columns over polyoxyethylenated cholesterol with approximately the same number of OE units.

At Figure 2, Chromatograms (1A and 1B) are obtained from two columns of equal dimensions (20 ft × 1/4") under the same operating conditions (column temp. 100 °C, inj. temp. 150 °C, det. temp. 150 °C, flow rate 4 ml/min. N_2, FID). The first column is packed with 10% Chol-7.37 (cholesterol oxyethylenates with 7.37 OE units) and the second column is packed with 10% PEG-300 (polyethylene glycol 300 i.e., with 6.80 OE units). It can be seen from these chromatograms that the two columns have the efficiency to resolve the solute pair completely, and more detectable retardation for the polar solute (1-propanol) on PEG-300 column than on Chol-7.37 indicating that glycol column is more polar than cholesterol oxyethylenate with the same average number of OE units.

At Figure 3, chromatograms (2A and 2B) obtained from Chol-33.90 and PEG-1500 columns which have 33.90 and 34.05 average number of OE units, respectively. These representative chromatograms give indication that glycol columns are more polar than cholesterol oxyethylenate ones which have approximately equal number of OE units. Careful inspection of the obtained chromatograms, shows that the retention time of the polar solute increases with increasing the number of OE units in glycol stationary phase, whereas, the retention time of the non polar solute remains almost constant. The same trend is observed when the examined solute pair is injected on cholesterol oxyethlenate stationary phases. Measured Δt, W_1, W_2 and calculated (R) values of stationary phase groups are listed in Table4.

It can be seen from Figure 4 that resolution values, of glycol and polyoxyethylenated cholesterol columns, increase with increasing the number of OE units and the rate of increase seems approximately the same and two parallel lines are obtained. The upper line represents the more polar PEG columns, and the lower line represents the less polar polyoxyethylenated cholesterol columns. The vertical distance between the two lines represents the difference in resolution (ΔR) between the two stationary phase groups. This resolution reduction is due to the influence of cholesteryl tail. The multiring system of the connected cholesteryl tail reduces the polarity of the polyoxyethylene (POE) head group, and hence the polarity of the whole surfactant molecule. This polarity reduction has its own influence on the retention data of the injected solute pair and subsequently on the obtained resolution (R) value Table 4.

Versatility of Column Packings

In the previous part of this gas-chromatographic application, the polarity of the prepared members of cholesterol oxyethylenates are estimated relative to the well-known polar carbowaxes (PEG) through resolution of polar and nonpolar solute pair. Symmetrical peaks and measurable peak widths are required to permit calculation of resolution values with the least amount of error.

Variety of column packings are well suited to separate mixtures of liquid organic compounds including wide variety of hydrocarbon-types, alcohols, chlorinated hydrocarbons and other solvent mixtures, ketones and cyclic ketones, aldehydes and esters. The prepared cholesterol oxyethylenate packings provide excellent resolution and sharp symmetrical peaks are obtained. Reasonable speed of analysis is achieved with 10% stationary phase loading. Versatility of column packings is shown from the following chromatograms:-

Figure 5 shows Chromatogram (3), 10% Chol-19.52, 12 ft × 1/8" stainless steel column provides a good separation of a mixture of cis- and trans isomers of 1,2-dichloroethylene along with 1,2-dichloroethane.

Figure 5 also shows Chromatogram (4), five pentanol isomers are separated on 10% Chol-14.75, 12 ft × 1/8" stainless steel column giving sharp symmetrical peaks without tailing in 7 minutes analysis time.

Figure 6 shows Chromatogram (5), Chol-19.52, 6 ft × 1/8" column provides base line separation for a solvent mixture of two ethers and two ketones in 3 minutes analysis time.

Figure 6 also shows Chromatogram (6), 10% Chol-14.75, 12 ft × 1/8" column separates iso-and n-C_4 and C_5 aldehydes along with 2-pentanol in 6 minutes analysis time.

Figure 7 shows Chromatogram (7), base line separation of four cyclic ketones without peak tailing on 10% chol-37.51, 24 ft column in less than 7 minutes analysis time.

Figure 7 also shows Chromatogram (8), 10% Chol-19.52, 18 ft × 1/8" stainless steel column provides excellent resolution for a mixture of toluene, ethylbenzene, n-propylbenzene along with methylcyclohexane.

CONCLUSION

1. When utilized as stationary phases, polyoxyethylenated cholesterol surfactants have proved to be less polar than polyethylene glycols (carbowaxes) having approximately the same OE units.
2. In polyoxyethylenated cholesterol non-ionic stationary phase surfactants, the polarity of the whole molecule is less than that of the POE head group alone. When the nonpolar multiring structure

of cholesterol is bonded to POE chain, through an ether linkage, the polarity of the latter is reduced.

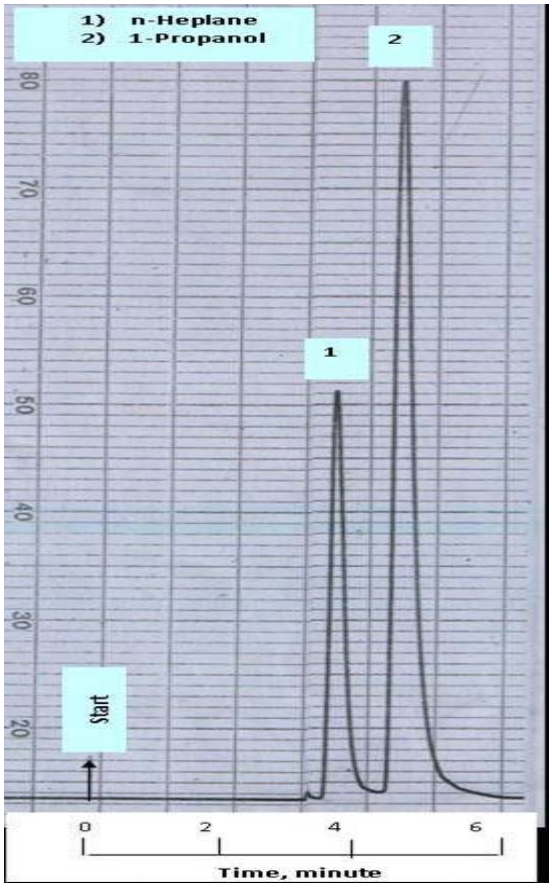

Figure 2: Chromatogram (1A) 10 % Chol- 7.37

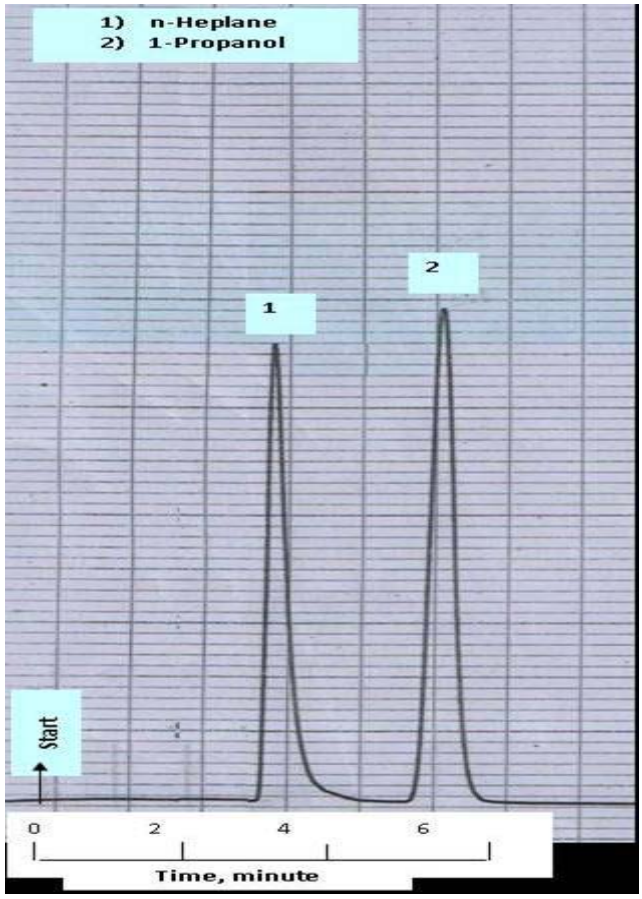

Figure 2: Chromatogram (1B)

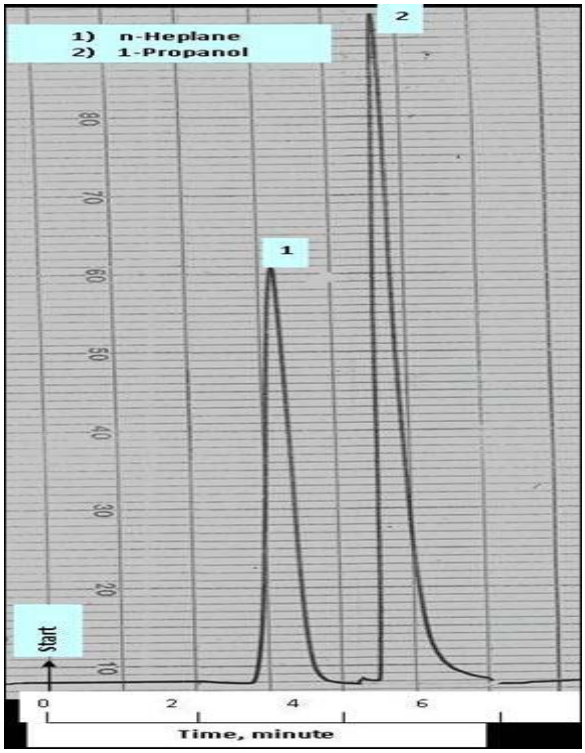

Figure 3: Chromatogram (2A) 10 % Chol- 33.9

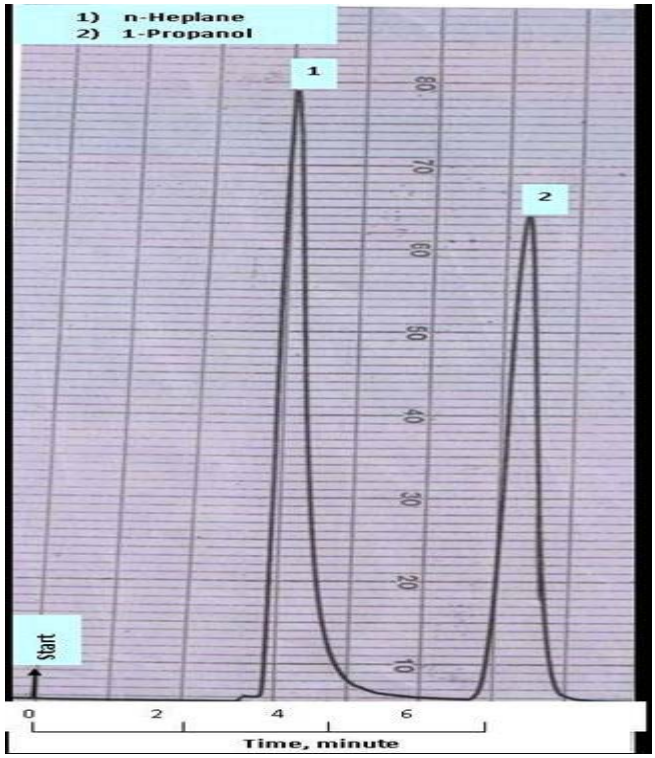

Figure 3: Chromatogram (2B) 10 % PEG- 1500 n=34.05

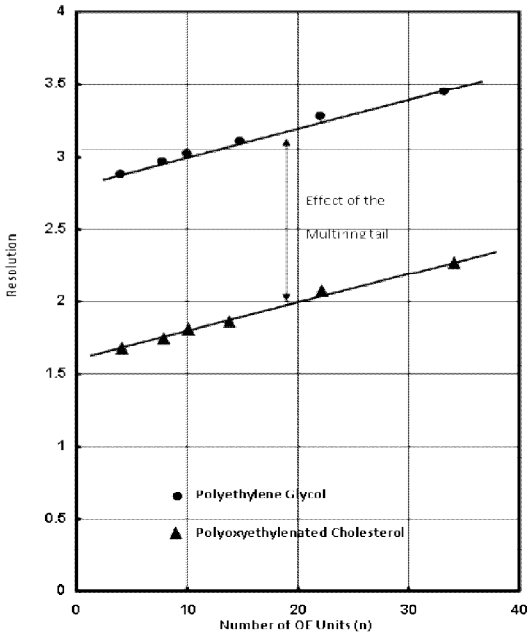

Figure 4: Resolution of a Solute Pair Versus OE Unit s on Polyoxyethylenated Cholesterol and Polyethylene Glycol Packed Columns

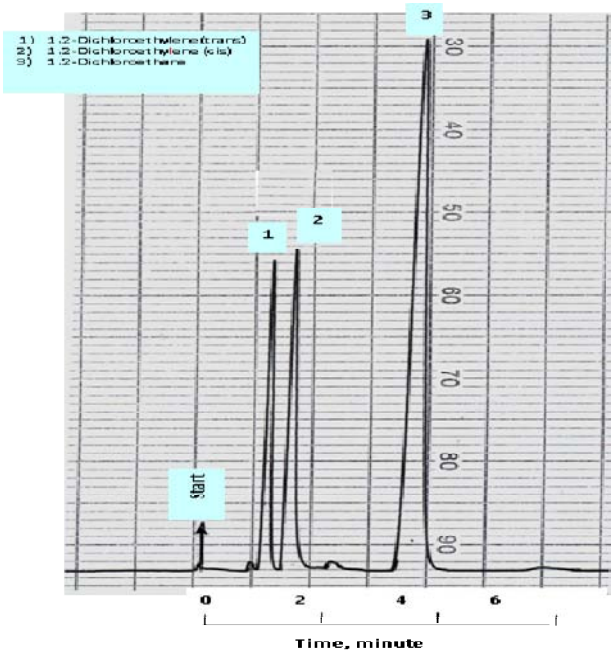

Figure 5: Chromatogram (3)

10 % Polyoxyethylenated Cholesterol with 13.87 OE units on 100 / 120 Chromosorb P AW-DMCS, 12 ft × 1/8"SS, Column Temperature 85 °C, Inj. Temp.: 135 °C, Det. Temp.:135 °C Flow Rate: 10 ml/min., N2, Det.: FID Sample 0.1 µl

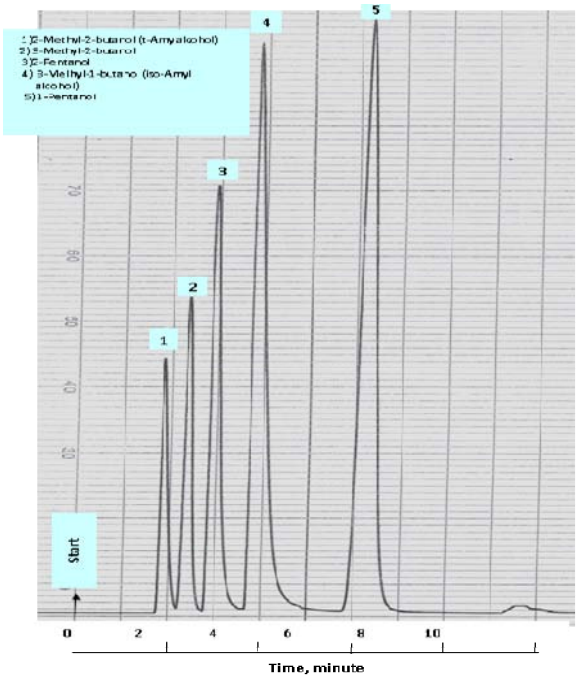

Figure 5: Chromatogram (4)

10 % Polyoxyethylenated Cholesterol with 13.87 OE units on 100 / 120 Chromosorb p AW-DMCS, 12 ft × 1/8"SS, Column Temp Temperature 120 °C, Inj .Temp: 170 °C, Det. Temp.: 170 °C Flow Rate: 18 ml/min., N2, Det.: FID, Sample 0.1 µl

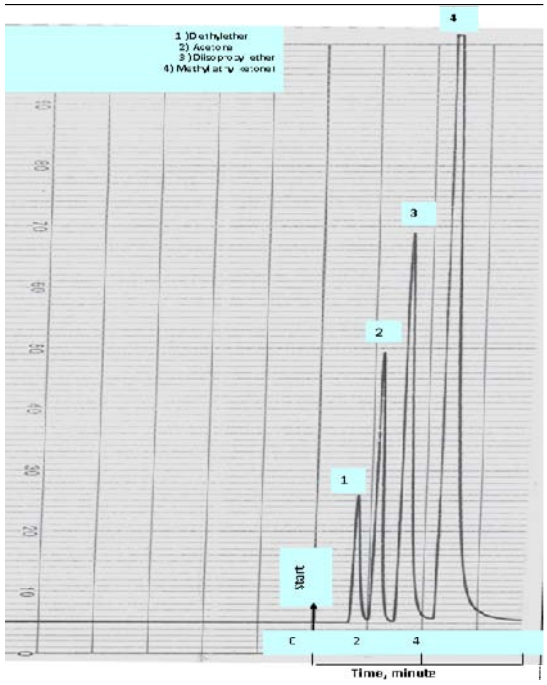

Figure 6: Chromatogram (5)

10 % Polyoxyethylenated Cholesterol with 13.87 OE units on 100 / 120 Chromosorb P AW-DMCS, 6 ft × 1/8" SS, Column Temperature 80 °C, Inj. Temp.: 120 °C, Det. Temp.: 120 °C; Flow Rate: 10 ml/min., N_2, Det.: FID, Sample. 1 µl

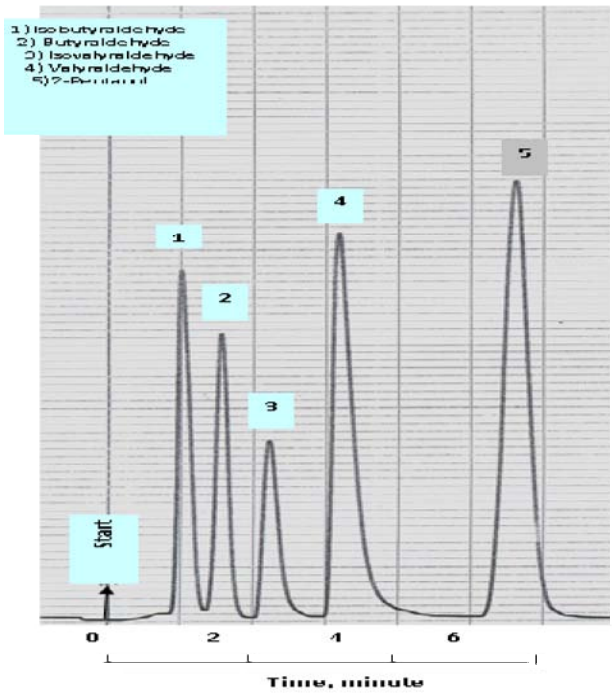

Figure 6: Chromatogram (6)

10 % Polyoxyethylenated Cholesterol with 13.87 OE units on 100 / 120 Chromosorb P AW-DMCS, 12 ft × 1/8" SS, Column Temperature 95 °C, Inj. Temp.: 145 °C, Det.; Temp.:145 °C Flow Rate: 15 ml/min., N2, Det.: FID, Sample 0.1 µl

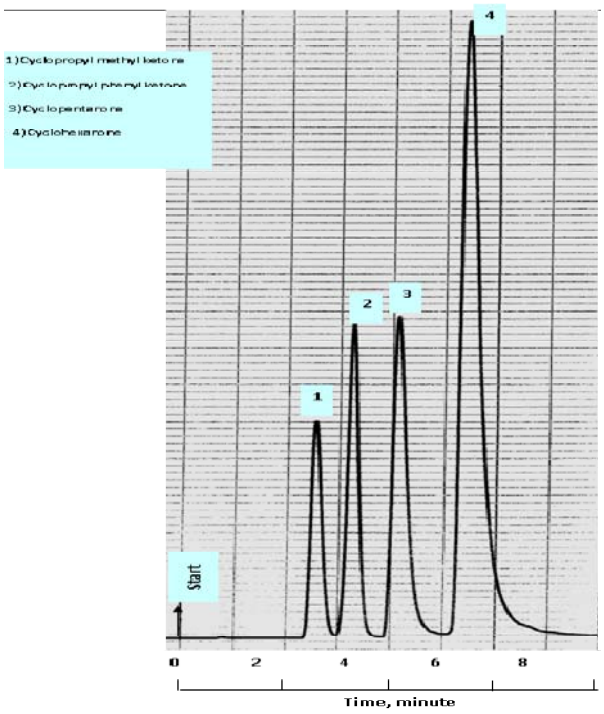

Figure 7: Chromatogram (7)

10 % Polyoxyethylenated Cholesterol with 33.90 OE units; on 100 / 120 Chromosorb P AW-DMCS, 24 ft × 1/8" SS; Column Temperature 130 °C, Inj. Temp.: 180 °C; Det. Temp.: 180 °C Flow Rate: 18 ml/min., N_2, Det.: FID; Sample. 1 µl.

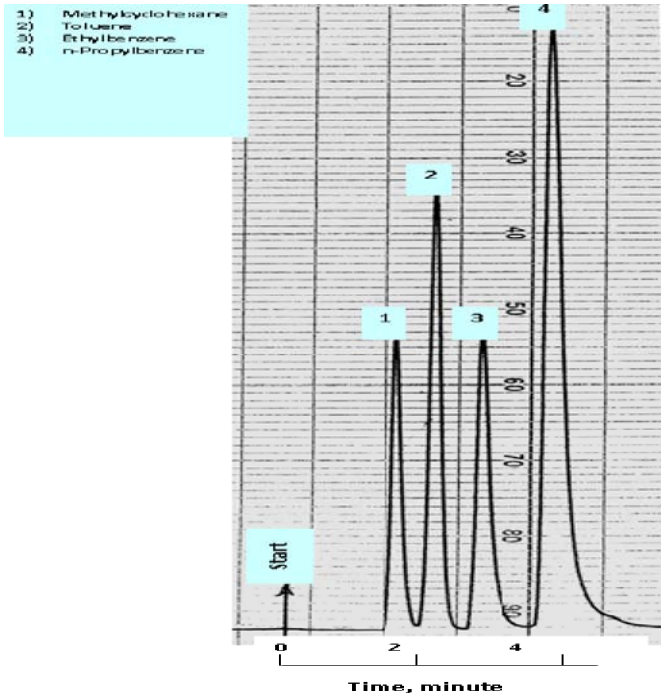

Figure 7: Chromatogram (8)

10 % Polyoxyethylenated Cholesterol with 23.17 OE; units on 100 / 120 Chromosorb P AW-DMCS, 18 ft × 1/8" SS; Column Temperature 145 °C, Inj. Temp.: 190 °C; Det. Temp.: 190 °C Flow Rate: 15 ml/min., N_2, Det.: FID; Sample. 1 µl.

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