

## Temperature as a factor in determining order of elution in gas-liquid chromatography of some C<sub>5</sub>-C<sub>8</sub> hydrocarbons

The usual practice in gas-liquid chromatography is to work at as low a column temperature as possible when seeking to increase the separation of close-boiling, chemically similar compounds. This is because, in many cases, the relative retention between two solutes increases with decreasing temperature. This view of the situation, however, is a simplified one. PURNELL<sup>1</sup> showed some years ago that, depending on the relative values of vapor pressure and the ratio of the molar heat of solution at infinite dilution to the molar latent heat of vaporization, increasing the column temperature may lead to either an increase or a decrease in resolution for a given pair of solutes. Thus, there may be both an upper and a lower column temperature at which the separation, in terms of the relative retentions, will be the same with a reversal in the order of elution.

Work in this laboratory, connected with the detailed analysis of hydrocarbon fractions isolated from a Fischer-Tropsch product<sup>2</sup>, revealed the existence of a complex relationship between the column temperature and the order of elution for some of these hydrocarbons. Fig. 1 is a plot of the logarithm of the retention for eight hydrocarbons, relative to hexane, over the temperature range 40° to 80°. Pure samples of the individual hydrocarbons admixed with hexane were chromatographed

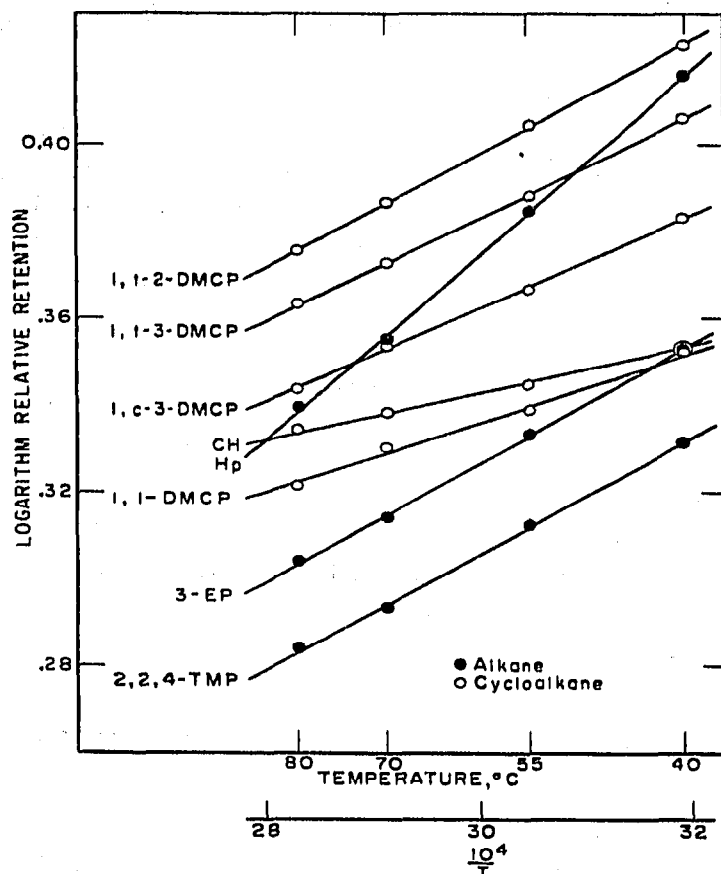


Fig. 1. Logarithm of retention, relative to hexane, as a function of temperature.

on a 26.5 ft. (4.6 mm I.D.) coiled column packed with 79 g of 12% (w/w) tricresyl phosphate on 34-45 mesh Chromosorb P. All the relative retentions are averages of duplicate measurements. Peak retention times were measured with a stopwatch starting from the emergence of the air peak. The helium flowrate was set at 72 c.c./min as measured by a soap-film flowmeter at room temperature.

The tricresyl phosphate used to prepare the column packing when analyzed by mass spectrometry using low-energy ionizing electrons<sup>3</sup> showed the following approximate distribution of molecular weights: 354, trace; 368 (tritoyl phosphate), 39%; 382, 43%; 396, 16%; and 410 (trixyl phosphate), 2%. Apparently this tricresyl phosphate was prepared from a cresol sample that contained appreciable xylenol. Other tricresyl phosphate samples, including one specifically labelled "for gas chromatography", when analyzed in this fashion showed similar molecular-weight distributions.

A marked change in the order of elution for the hydrocarbons occurs as the column temperature changes. At 40°, the order of elution is 2,2,4-trimethylpentane (2,2,4-TMP), 1,1-dimethylcyclopentane (1,1-DMCP), 3-ethylpentane (3-EP) and cyclohexane (CH), 1-cis-3-dimethylcyclopentane (1-c-3-DMCP), 1-trans-3-dimethylcyclopentane (1-t-3-DMCP), heptane (Hp), and 1-trans-2-dimethylcyclopentane (1-t-2-DMCP). At 80°, the order of elution is 2,2,4-TMP, 3-EP, 1,1-DMCP, CH, Hp, 1-c-3-DMCP, 1-t-3-DMCP, and 1-t-2-DMCP.

For the pair methylcyclohexane-1-cis-2-dimethylcyclopentane the relative retention increases from 1.03<sub>4</sub> at 40°, where they are not separable, to 1.05<sub>3</sub> at 80°, where they could be separated sufficiently for analysis. Again, on the same column, the relative retention for the pair cyclopentane-hexane changes from 0.95<sub>4</sub> at 40° to 1.03<sub>3</sub> at 80°.

It is not generally recognized that isolated results showing changes in order of elution with changing column temperature have been reported in several papers. EVERED AND POLLARD<sup>4</sup> showed that 1-nitrobutane, when chromatographed on a squalane-coated packing, elutes before *n*-butyl nitrate at 80°, but that at 139° the order of elution is reversed. SIMMONS *et al.*<sup>5</sup> showed a reversal in order of elution on a squalane-coated capillary between 46° and 106° for the pair 2,2-dimethyl-3-ethylpentane-2,6-dimethylheptane. They also obtained a slightly better separation between 2,5- and 3,5-dimethylheptane at the higher temperature. ROBERTS<sup>6</sup> showed that caryophyllene and farnesene exhibit a reversal in order of elution between 100° and 200° on an Apiezon L packing. BARON AND MAUME<sup>7</sup> reported that several terpene alcohols undergo reversal in order of elution between 130° and 150° when chromatographed on a Reoplex 400 packing. SCOTT<sup>8</sup>, in a detailed study of dinonyl phthalate-coated capillary columns, demonstrated that the pair methylcyclohexane-heptane is more easily resolved at 60° than at lower temperatures, partly because the retention ratio increases in going from 0° to 70°. DESTY AND GOLDUP<sup>9</sup> reported better resolution of the octanes on a squalane-coated capillary at 50° than at lower column temperatures.

Thus, various solutes, on a variety of columns, exhibit complicated elution behavior when the column temperature is changed. This may be, therefore, a phenomenon of general interest. As more chromatographic systems are studied in detail, it is to be expected that further examples of this type of behavior will be found. It may be helpful in anticipating other examples to point out that in many cases, both

in this paper (Hp and the DMCP's in Fig. 1, cyclopentane-hexane, and methylcyclohexane-1-cis-2-DMCP) and in the literature<sup>6-8</sup>, this phenomenon occurred with pairs of compounds consisting of either acyclic and cyclic structures or different ring structures.

Consequently, for solutes that elute close together, column temperature may often be a factor in determining order of elution. The order of elution established experimentally at one temperature may not hold true at a different column temperature, particularly for multicomponent mixtures. In addition, in programmed-temperature gas chromatography, the following situations may arise. If the column temperature is programmed during the run, a separation that would have been achieved at the lower temperature may be partly or wholly canceled out by operating at a higher temperature where a reversal in the order of elution occurs. For compounds eluting close together, the order of elution may depend on the choice of initial and final column temperatures or on the rate of heating of the temperature program.

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### **The influence of esterifying and acetylating groups on the retention times of amino acid derivatives in gas chromatography**

A number of publications have appeared recently reporting studies on the analysis of amino acid mixtures by gas phase chromatographic separation of their more volatile derivatives. In particular the esters<sup>1,2</sup>, N-trifluoroacetylated esters<sup>3-8</sup>, and N-acetylated esters<sup>9,10</sup> have attracted considerable attention. Each of these reports have in

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