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Elution of oxygen from a highly activated molecular sieve 5A column

It has been shown[1,2] that a gas chromatograph using a highly activated molecular sieve type 5A column can effect the separation of oxygen and argon when present together as trace impurities in helium. Since we are concerned with the analysis of high purity gases, including helium, it was decided to investigate the utility of a highly activated molecular sieve type 5A column for the determination of these trace impurities. The gas chromatograph was similar to that previously described[3], except that a glass tube was used to contain the molecular sieve material. The column material was activated, *in situ*, at a temperature of 400° for a period of 72 h whilst being purged continuously with purified helium at a flow rate of 20 ml/min. Preliminary tests confirmed the separation of oxygen and argon, but showed that the retention time for oxygen was dependent on the sample size (*i.e.* 1 ml or 10 ml) and/or the concentration of oxygen in the sample. This effect was more noticeable when the column was operated at ambient temperature than at 80°.

To verify these observations, experiments were conducted using a highly activated column at two different temperatures (*i.e.* 28° and 80°). Ten milliliter aliquots of helium containing oxygen at different "v.p.m. levels" were prepared, using a dynamic gas blending rig[4], and analyzed. The peak heights and retention times were then measured for each analysis (see Table I). The results show a large

TABLE I

RETENTION TIME OF OXYGEN AS A FUNCTION OF OXYGEN CONCENTRATION IN A 10 ml SAMPLE OF HELIUM

Columns, 30–60 mesh molecular sieve 5A. Flowrate of helium carrier gas approx. 75 ml/min.

Column activation temperature (°C)	*Column operating temperature* (°C)	*Oxygen* (*v.p.m.*)	*Peak height* (10^{-9} *A*)	*Retention time* (*min*)
400 (for 72 h) highly activated	28	78	77.0	1.64
		51	52.8	1.70
		25	18.8	1.64
		12	3.2	2.20
		6	0.50	3.00
		2.6	0.12	5.40
400 (for 72 h) highly activated	80	78	82.0	0.96
		51	66.0	0.96
		25	38.8	0.94
		12	15.6	1.00
		6	5.7	1.02
		2.6	1.1	1.12
300 (for 24 h) normally activated	28	74	77.3	1.40
		51	62.3	1.36
		25	37.3	1.40
		12	15.5	1.44
		6	7.2	1.42
		2.6	2.7	1.40

increase in retention time as the oxygen concentration of the sample is reduced with a column temperature of 28°, and a much smaller increase with a column temperature of 80°; the retention times were measured from the oxygen peak maximum. A molecular sieve column activated at 300° did not show any change in retention time with concentration (Table I).

It is apparent therefore that at levels below about 10 v.p.m. of oxygen using a highly activated column at room temperature it is possible for oxygen to elute at the same time as nitrogen (or even methane), and hence careful calibration using oxygen in helium mixtures is essential.

A special blend of helium was prepared containing 12 v.p.m. each of hydrogen, argon, nitrogen and methane, and 23 v.p.m. of oxygen and was contained in a gas cylinder at 1000 p.s.i.g. Its composition was verified by mass spectrometric analysis[4]. Chromatograms (Fig. 1) for a 1 ml and a 10 ml sample of this blend shows the dependence of the retention time for oxygen on the sample size. The "anomalous" response of the helium ionisation detector, giving rise to negative peaks for hydrogen, argon and nitrogen has been described in an earlier paper[3].

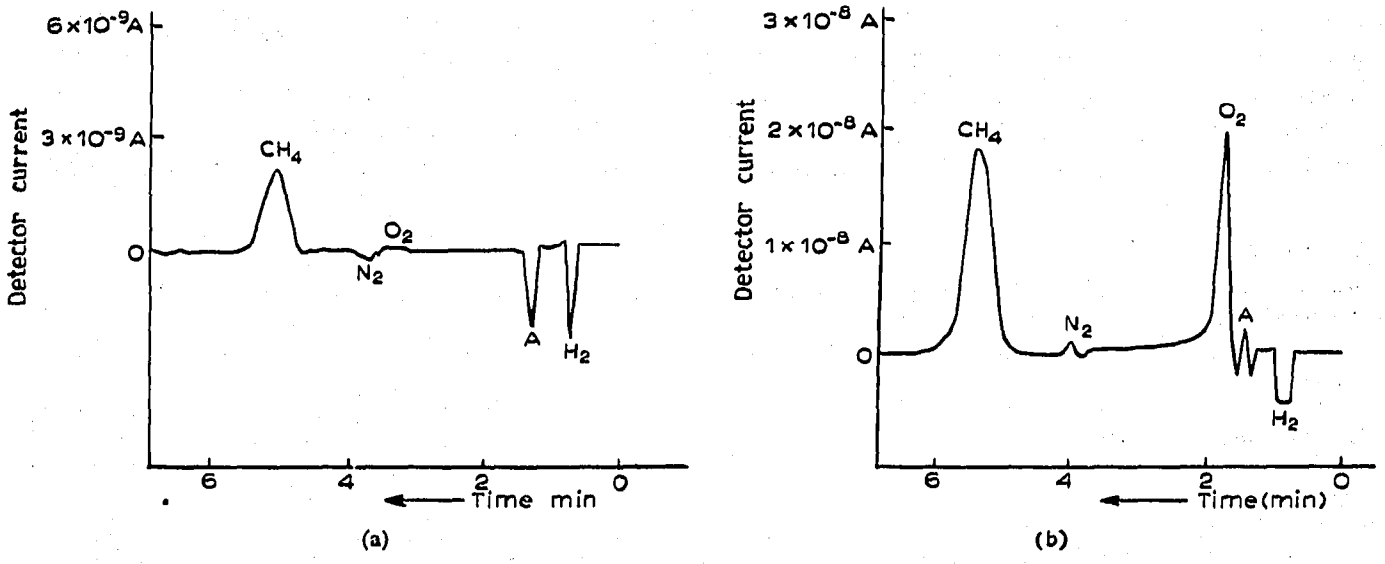

Fig. 1. Typical chromatograms of helium blend. (a) = 1 ml sample; (b) = 10 ml sample.

WALKER[1] found that the resolution of the oxygen and argon peaks deteriorated slightly over a period of 5 days. This was not confirmed in the present work, and our experience has shown that separation of oxygen and argon can still be achieved after injection of approximately one hundred 10 ml samples of the above gas mixture over a period of four weeks with no apparent change in the retention times of the oxygen and argon. These tests were carried out at a column temperature of 28°, with gas samples which were relatively dry.

The effect of variation in retention time with concentration of component is not unknown, and a similar effect with nitric oxide on a molecular sieve 5A column has been described[5]. In order to check whether "conditioning" of the highly activated column would affect the retention time, five 10 ml samples of pure oxygen were injected into the column (operating at 28°), and after a stabilisation period of 30 min, the gas mixture contained in the cylinder was analysed at intervals using both 1 ml

and 10 ml samples. Initially, for both sample sizes, there was an increase in sensitivity. However, during a period of 2 h, the oxygen sensitivity fell back to its original level. It would appear, therefore, that either "conditioning" with pure oxygen had not been extensive enough, or that the column requires "conditioning" before each sample injection.

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Biochemistry of sphingolipids

XXIII. Paper chromatography of human brain gangliosides

Gangliosides represent a complex group of glycosphingolipids characterized by the presence of N-acetylneuraminic acid in the carbohydrate chain. They occur primarily in brain tissue, but are now known to exist outside the central nervous system as well (*e.g.* spleen, erythrocytes etc.) Alterations of their pattern in certain neurological diseases has provided additional impetus to the study of their chemistry and metabolism.

The separation of gangliosides has recently been achieved through the use of thin-layer chromatography. The heterogeneity of these substances in chromatographic systems has been well documented; however, the number of individual types and their chemical composition reported by various authors varies[1–7]. An excellent comparative thin-layer chromatographic study has been published recently by PENICK *et al.*[8]

Only a few authors have reported the separation of gangliosides on paper, so far. SVENNERHOLM[5,9] describes some solvent systems including tetrahydrofuran–diisobutyl ketone–water, diisobutyl ketone–acetic acid–water and *n*-butanol–pyridine–water mixtures for the separation of these compounds. MICHALEC AND KOLMAN[10] used Schleicher and Schüll No. 289 silica gel paper and several solvent systems for the one- or two-dimensional chromatography of gangliosides.

During our study on the brain ganglioside spectrum, we have applied paper