NEGATIVE PEAKS IN GAS CHROMATOGRAPHY: VACANCY CHROMATOGRAPHY AS THE CAUSE OF ANOMALOUS RESPONSE OF THERMAL CONDUCTIVITY DETECTORS

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While other new detectors are continuously introduced, the thermal conductivity detector (TCD) continues to be widely used for many applications[10].

This detector is in general use for the analysis of inorganic gases, to which the ionization detectors are often insensitive. Mixtures of organic and inorganic compounds must also be analyzed by TCD.

To achieve accurate results, one must pay great attention to the choice of the most suitable carrier gas. Generally speaking, hydrogen and helium are the most widely used; nitrogen, argon, carbon dioxide and others can also be employed for given separations[11]. Also binary mixtures of gases have been used to linearize the response of TCD[7], but, notwithstanding the several attempts made to obtain an ideal carrier gas, some anomalous results are often reported.

Negative peaks—*i.e.* peaks opposite in polarity to those normally recorded in a given separation—are an example of such anomalous response. In many cases, these negative peaks can be explained as depending on the geometry of the thermal conductivity cell, the filament temperature and operating conditions. In other cases, especially those with the so-called "M" of "W" shaped peaks, the anomalous response is due to the fact that, in gaseous mixtures, thermal conductivity is not a linear function of concentration but shows maxima and minima.

This phenomenon may be observed not only when a gas mixture is used as carrier, but also when the mixture is formed in the detector cell by the carrier gas and by the compounds eluted from the column[2,3,6,8,9,13]. A typical example is given by the helium–hydrogen mixture[4]. When peak inversion is due to the described phenomenon, the "W" shape of the peak is always present, almost in any range of sample quantity.

Another type of negative peak, which cannot be explained by the non-linearity theory, has been observed, especially in trace analysis. This anomalous response differs from those described before, because it never shows "W" shaped peaks, but the passage from positive to negative peaks, when the sample quantity decreases, is simply a "zero-signal"—*i.e.* the baseline does not show any appreciable deflection. The phenomenon is thus a "total inversion".

At the "inversion point" one can argue that a given component will be absent from the injected sample, and this may produce serious errors in the analytical

result. In the same manner, the quantitative values obtained from peak areas close to the inversion point are incorrect.

In the present work the total inversion phenomenon is described, and a study of its causes and of possible correction methods is made.

NATURE OF THE INVERSION PHENOMENON

Total inversion of peaks may be encountered during testing for purity. This can happen when one analyzes a slightly impure gas by TCD, and a negative peak appears, with a retention time equal to that of impurity. Decreasing the impurity concentration in the sample, the area of the negative peak increases to a maximum for a completely pure sample; on the contrary, on increasing the concentration of the impurity in the sample, the "negative area" decreases in absolute value until the "inversion point" where the peak disappears. After this point, an increase of the impurity concentration is recorded as an increasing positive peak.

Fig. 1 shows the peaks obtained on analyzing increasing concentrations of N_2 in helium with hydrogen as carrier gas. (Only the nitrogen peaks are shown.)

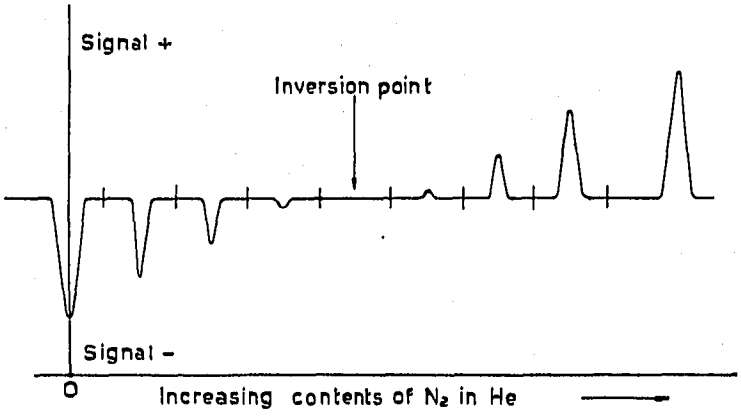


Fig. 1. Analysis of increasing small amounts of nitrogen with the thermal conductivity detector. Hydrogen with traces of nitrogen as carrier gas (only the nitrogen peaks are shown).

In Fig. 2 (solid line) the recorded areas S_p are plotted against the theoretical areas S_t, which are expected from the injection of known amounts of nitrogen (the dotted line represents the theoretical behaviour, with zero signal at zero nitrogen content). As can be seen, considerable error is introduced near the inversion point (I.P.). No value is given on the plot, because the position of the inversion point may correspond to any value of S_t, *i.e.* any value of nitrogen. The inversion point position is controlled by the purity of the carrier gas. The dotted line in Fig. 2 shows the plot of S_p *vs.* S_t obtained with less pure hydrogen as carrier gas.

The anomalous behaviour is shown with every combination of the three elements: carrier—sample—impurity.

AHUJA *et al.*[1] describe the phenomenon and suggest that the negative peak is formed when the liquid phase dissolves a component present in the carrier gas and yields it to injected sample.

Other authors[12] explain negative peak occurrence with flame ionization destector

as due to trace impurities in the carrier gas, which produce the so-called "vacancy chromatography".

When an impure carrier gas is flowing in the gas chromatographic column, the contaminants are dissolved in the stationary phase, and are retarded in their progress through the column, depending on their retention times. Thus several streams of different gases travel at different speeds in the column. In the steady-state condition of the system, the composition of eluted carrier is constant and the signal is zero.

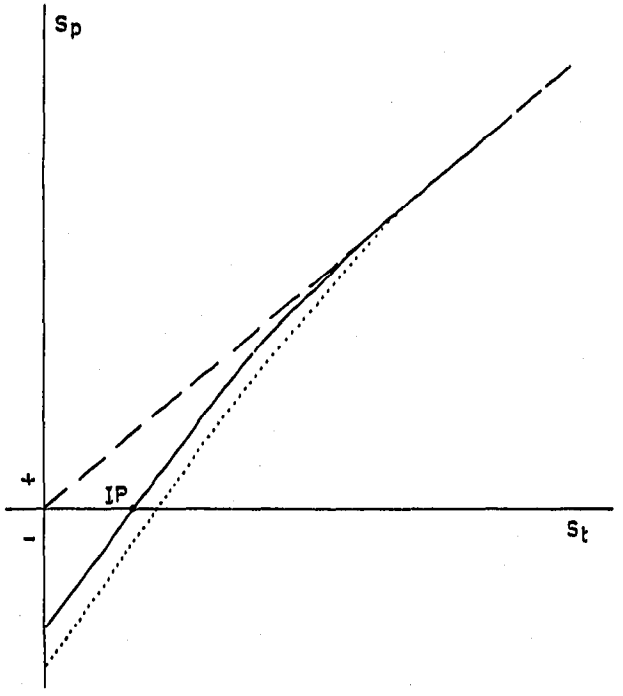


Fig. 2. Nitrogen peak areas recorded (S_p) *vs.* areas expected (S_t) with pure and nitrogen containing carrier gas. (— — —) pure carrier gas; (————) impure carrier gas; (- - -) more impure carrier gas.

With the TC detector this means that the gas flowing through the analysis side of detector has the same composition as that flowing through the reference side, and the bridge is balanced.

With the flame ionization detector the resulting "zero" signal is the sum of the currents developed by the combustible contaminating components plus a minimum signal developed by the hydrogen combustion. This constant signal may be bucked out, and, since there is no interruption or change of flow, there is no change in the signal, and no peaks are shown. The introduction of a pure sample produces a change in composition of the gas stream, and causes a "hole" (or impurity free zone) in the stream of every contaminant. Each "hole" travels through the column with a speed depending on the retention time of the corresponding contaminant.

When a low-concentration zone, or vacancy, enters the detector, a negative peak is formed.

If a flame ionization detector is used, negative peaks are produced only by combustible compounds and are due to decreasing background current in the low

concentration zone. Using alternatively clean and contaminated gases as carrier or sample, a negative and a positive chromatogram corresponding perfectly are formed[12].

Such a phenomenon was also observed with EC detectors, and negative peaks are due to the increase of "standing current" when the clean carrier gas of the vacancies enters the space between the anode and the radioactive source. This is noticeable especially when the carrier gas is not completely dry and a negative peak corresponding to the retention time of water is observed[1].

The helium detector[5] is particularly sensitive to impurities in the carrier gas, and negative peaks due to vacancies are observed.

The "absolute" nature of ionization detectors makes the study of the "total inversion" phenomenon easier than in the case of the "relative" TC detector. In the latter case, one must also take into account the gas stream which flows on the reference side of the TC detector.

EXPERIMENTAL

As can be seen from Fig. 3, only with a dual column gas chromatograph with separate flow regulation on the two columns (or with special features in a single column instrument), it is possible to achieve a perfect matching between the instantaneous contents of carrier gas in the two detector sides.

A special apparatus (shown in Fig. 4) was developed to evaluate the effects of carrier purity and other conditions on the "total inversion" phenomenon.

With the flow path shown as a solid line it was possible to reproduce conditions (B) and (C) of Fig. 3. With a flow modification (dashed line) and on-off valves (not shown in figure) one could also reproduce condition (A).

Traps T_1 and T_2 (filled with Molecular Sieve 5 A and cooled with Dewar flasks containing liquid nitrogen) and by-pass systems allowed cooling of the carrier stream or the sample stream, or both. According to data by HARTMANN AND DIMICK[5], cooling gave several liters of pure gas, before trap regeneration was necessary.

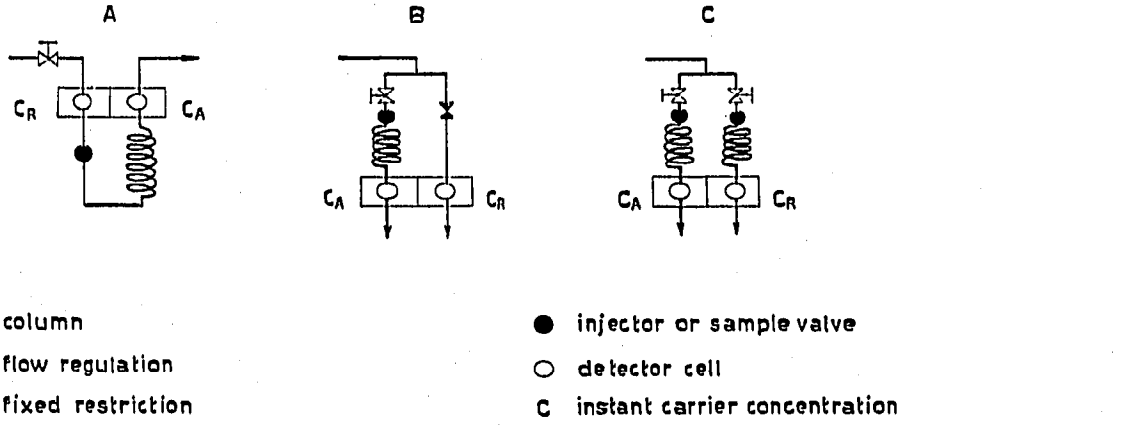
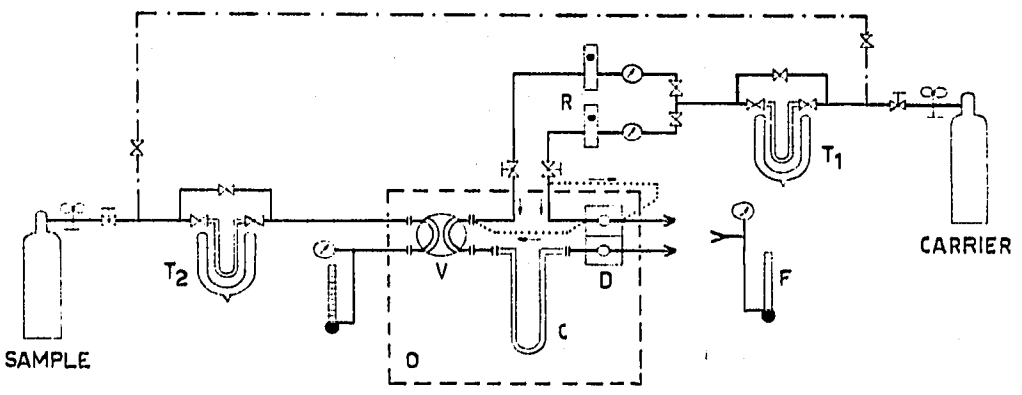


Fig. 3. Different carrier gas flow paths in three common types of chromatographic instruments. In (A) C_A and C_R always differ; in (B) C_A and C_R generally differ; in (C) C_A and C_R may easily be equal.



⋈	on-off valve	F	bubble flowmeter
⋈	flow metering valve	O	oven - thermostat
⊘	pressure gauge	R	rotameters
C	column	T	molecular sieve trap
D	detector	V	gas sample valve

Fig. 4. Apparatus designed to control variable parameters of gas chromatographic analysis.

EFFECTS OF CARRIER GAS PURITY

The following experiment was carried out in order to study the effect of carrier gas purity.

The two trap systems were supplied with the same hydrogen containing a low percentage of nitrogen and oxygen (symbol H_x), and the column was filled with Molecular Sieve 5 A. The resulting chromatograms are shown in Fig. 5 (where notation H/H represents the pair sample/carrier of every analysis). Depending on the experimental conditions we obtained the following results:

(1) *The pressures and the gas streams on the analysis and reference sides of the TCD are perfectly equal (i.e., the carrier gas quantities C_R and C_A are equivalent)*

In this case no analysis peak is shown when a sample is introduced (1a) because one simply replaces a given amount of carrier gas with an equal amount of the

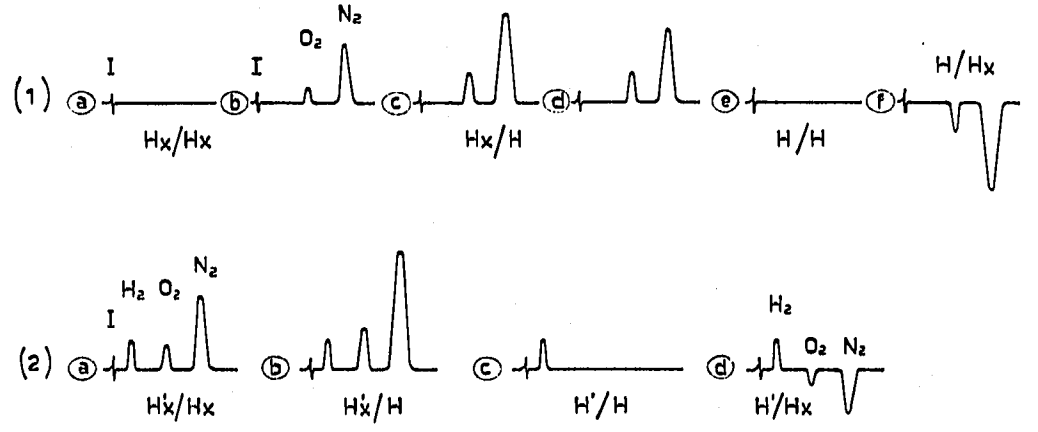


Fig. 5. Analysis of impure hydrogen with the thermal conductivity detector and the same hydrogen as carrier gas. The sample and/or the carrier gas are alternatively purified by cooled traps. (1) The concentrations of carrier and sample gas in the two detector sides are equal. (2) The concentration on the analysis side is greater.

same gas. Peak I is due to instantaneous flow interruption in sampling[3]. When T_1 is cooled, the impurities present in the carrier flow are removed, the carrier is pure hydrogen H and positive peaks appear.

Obviously, as the cooling trap produces a variation in flow, it is necessary that the equivalence of the concentrations C_R and C_A is maintained by means of adequate regulations.

On the other hand, if only T_2 is cooled and the sample stream purified, the series of chromatograms obtained is a perfect inversion of that described previously. The chromatogram (1f) of Fig. 5 shows the maximum areas of the peaks, which are equal in absolute value to those of chromatogram (1c).

This series of experiments demonstrates that the total inversion phenomenon is shown when the carrier is less pure than the sample.

(2) *If pressures and flows on the two sides of the detector are different (i.e. if C_R and C_A differ)*

In this case, the series of chromatograms shown in Fig. 5-2 is formed, assuming C_A greater than C_R (H_x' is the sample, at a flow greater than those of the carrier H_x, having the same composition. (The notation is the same as in Fig. 5-1; H' and H indicate the purified hydrogen streams).

In the chromatograms of series 2, the hydrogen peak is present, while in series 1 it was not observed. This is due to the fact that one replaces a given amount of carrier gas with a larger quantity of the same gas. In 2a, oxygen and nitrogen peaks are also present, and they are positive as the absolute quantity of each impurity is greater in the sample than in the carrier.

On cooling trap T_1, the carrier is purified and the peaks increase (2b). The maximum is shown.

On cooling both traps, the sample and the carrier are formed by pure gas, and only the difference in concentration on the two detector sides produces the hydrogen peak.

On cooling only trap T_2, the sample is purified and negative peaks are formed for oxygen, and nitrogen, while hydrogen produces a positive peak. By calculating the areas of oxygen and nitrogen peaks, one can see that the values in (2a) are the difference between the values in (2b) and (2d).

If C_R is greater than C_A the described phenomena are completely reversed. A general relation can be derived from these experiments.

It is well known that the area of the peak is given by the formula:

$$S = n/fm \qquad (1)$$

where:

S = peak area
n = moles of compound
fm = molar correction factor characteristic of every sample–carrier pair.

If the compound is present with concentration x in the carrier gas, n is expressed by:

$$n = xC \qquad (2)$$

where:

C = moles of carrier gas in the detector cell.

If C_A and C_R are different (due to differences in pressure in the detector cells), and the compound is present in different concentrations x' and x'', and the following general equation can be written:

$$S = \frac{x'C_A - x''C_R}{fm} \tag{3}$$

which is valid for every compound and carrier gas.

In Fig. 5 we have observed the results of such a formula. In fact, while the hydrogen area, if present, is constant, because $x\ H_2 = 1$ and $fm\ H_2/H_2 = 1$, the impurity areas are proportional to the fm of the oxygen/hydrogen and nitrogen/hydrogen pairs, and to the values $(C_A - C_R)$, C_A and $(-C_R)$ respectively in 2a, 2b and 2d.

Formula (3) is also valid when the major component of the sample is a gas different from the carrier.

Analyzing helium with x content of nitrogen on hydrogen with the same nitrogen content, a series of chromatograms similar to those of Fig. 5 was obtained. Obviously the helium peak was always present, and the impurity peak disappeared only when the sample and carrier pressures, flows etc. were adjusted to cause the same amount of impurity to be present in the two detector sides.

APPARATUS GEOMETRY AND OTHER PARAMETERS

The apparatus described in Fig. 4 was designed to give a complete knowledge and control of the parameters of analysis: temperature, pressure, flow, etc.

Commercially available gas-chromatographic instruments do not have all these facilities, and some cases of peak inversion can result from the particular construction of apparatus.

For instance, as derived from formula 3 and seen experimentally, the flow path (A) of Fig. 3 produces negative impurity peaks when the impurity concentrations are the same in the sample and in the carrier gas. In fact, equal partial pressure of impurity in the two detector sides does not result in equal absolute amounts, due to column backpressure. In this case, when the carrier gas contains impurities, analysis of samples with larger contents of the same compounds, may produce no peak, with large errors in their determination.

On the other hand, instruments having flow path (B), or, better, (C), may easily be regulated to have the same pressure and flow in the two sides of the detector.

The structure of the sample introduction system must also be considered. If a gas sampling valve is used, which replaces a given volume of carrier with an equal volume of sample, factors such as pressure, flow, viscosity, density of the two gas streams influence the results. With the syringe injection technique, the evaporation of relatively large amounts of liquid in a small volume may also produce changes in flow and pressure.

The nature of the stationary phase does not play a fundamental role in the phenomenon, which depends mainly on the carrier gas purity. A slight effect can be produced when the liquid phase dissolves or the support absorbs a certain amount of the impurities present in the carrier gas, and a concentration equilibrium is established.

When a vacancy travels through the column, the stationary phase, in contact with clean gas, yields to it the impurity corresponding to the vacancy. The area of the resulting peak is still negative, but smaller in absolute value than the area that appears when the described phenomenon does not happen.

CARRIER GAS PURIFICATION

The vacancy chromatography phenomenon can be avoided using a completely pure carrier gas.

The commercially available "pure grade" gases are expensive and may be too contaminated for satisfactory analyses, especially when traces must be determined.

Cleaning of the carrier gas with Molecular Sieve and anhydrone traps at the temperature of liquid nitrogen is the best method of obtaining a good purity.

If the gas supplied is not too contaminated, a large trap will easily last eight hours before regeneration at 300° is necessary.

CORRECTION METHOD

In many cases, carrier gas purification may be considered too expensive, or difficult to realise. It is possible to use a slightly contaminated carrier gas, and to have good results, if a correction value is determined.

From eqn. (3) it can be derived that, when all the experimental conditions are maintained constant, the peak corresponding to a compound simultaneously present in the carrier gas and in the sample has an area equal to the algebraic sum of the area produced by the analysis of the sample on clean carrier gas plus the negative area produced by the vacancy phenomenon (Fig. 6). The area experimentally produced, S_p, may be negative, zero or positive, depending on the absolute values of the expected sample area S_t and of the vacancy area S_v.

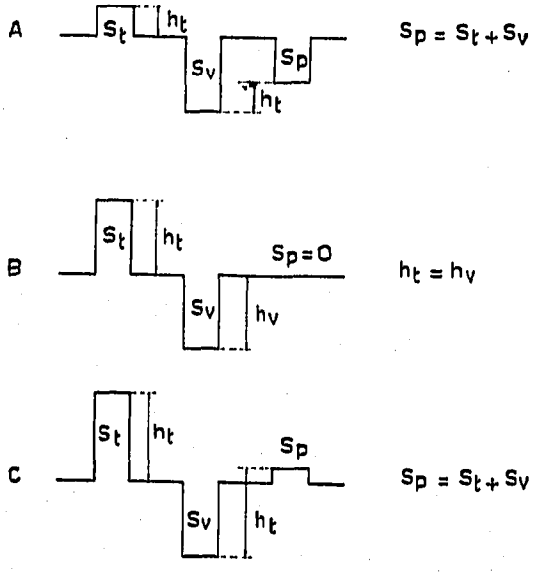


Fig. 6. Experimentally recorded peak area S_p as a sum of expected area S_t and vacancy area S_v, when the carrier gas contains an impurity.

To determine the correction value, *i.e.* the vacancy area S_v, it is necessary to inject a sample of completely pure gas under the same experimental conditions as those of the samples analysed.

The pure gas may be prepared by the same technique used for carrier gas purification, *i.e.* with cooled traps, but it is important to note that only a small trap is necessary for a short time.

With the apparatus shown in Fig. 4 we have controlled the method, using helium and hydrogen with known amounts of nitrogen as carrier and sample gases respectively.

Table I shows the average areas of the nitrogen peak obtained on analyzing impure hydrogen on impure helium (H/He) or cooling T_2 or T_1 (respectively H_p/He and H/He_p, where p indicates the purified gas). The values obtained by subtracting S_v from S_p are in good agreement with those obtained by analyzing the sample in purified helium (S_t).

TABLE I

COMPARISON OF RESULTS OBTAINED BY THE "CORRECTION VALUE" METHOD AND BY CARRIER GAS PURIFICATION

The areas of the nitrogen peak obtained with pure helium He_p as carrier gas (S_t) and those obtained by difference ($S_p - S_v$) between practical peak and vacancy peak are reported.

	H/He S_p	*H_p/He* S_v	$S_p - S_v$	*H/He_p* S_t	% *Deviation*
1	— 468	—1027	+ 559	+ 563	+0.71
2	+ 230	—1028	+1258	+1250	—0.64
3	— 205	— 942	+ 737	+ 730	—0.96
4	+1320	— 950	+2270	+2281	+0.48
5	— 108	— 430	+ 322	+ 327	+1.55
6	+ 603	— 442	+1045	+1053	+0.76

In every analysis the sample amount was different, and was selected in order to give a negative and a positive S_p for each of the three different carrier flows. The differences in S_v in the analyses carried out with the same carrier flow (1 and 2, 3 and 4, 5 and 6) are due to the fact that after every set of analyses, the trap T_2 was allowed to warm up to ambient temperature, and afterwards cooled to establish the S_v for the new series of injections. The difference was very slight, and this means that the trap was not yet saturated.

The percentage deviation is low, but increases as the peak area decreases. It is not clear if this is due to errors in area measurements or to other reasons.

CONCLUSIONS

The experiments described have shown that the total peak inversion is due to contaminated carrier gas and to vacancy chromatography phenomena, in the case of both thermal conductivity detector and ionization type detectors.

Every impurity of the carrier gas, if separately eluted from the column,

produces in the chromatogram a peak, the area of which is proportional to difference of the absolute concentration of the substance in the two sides of the detector.

If absolute concentrations are equal, no peak is shown.

Carrier gas purification, with refrigerated traps, causes the total inversion phenomenon to disappear.

As carrier gas purification may sometimes be difficult, a correction system based on purification of a small amount of gas is convenient and precise enough for practical purposes.

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SUMMARY

It was shown that the anomalous negative peaks which can appear in the gas chromatographic analysis of very small amounts of sample, are due to slightly impure carrier gas and to the "vacancy chromatography" phenomenon. The subsequent error is larger when the sample amount is smaller.

As the commercially available gases are never completely pure, two correction methods were examined:

(a) purification of all the carrier gas,

(b) a correction system based on purification of a small amount of the carrier gas.

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