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Ensemble Averaging in Gas Chromatography

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Abstract

Ensemble averaging has been successfully applied in gas chromatography. The normally accepted detection limit of methane for the flame ionization detector has been lowered by about two orders of magnitude. Analytically useful data were obtained when the signal-to-noise ratio of the generated ensemble was greater than two. The technique should be valuable not only in gas chromatographic determinations of ultra-trace constituents, but also in fundamental chromatographic studies involving very dilute samples.

Considerable enhancement of the signal-to-noise ratio for small repetitive signals buried in large amounts of random noise can be achieved by averaging the response of successive measurements. Such a technique, frequently described as ensemble averaging, is a widely accepted method for handling small, noisy responses from detectors, and has been applied in many forms of spectroscopy and electrochemistry. A review by Fisher (1) discussed the general instrumental requirements necessary for the successful application of this technique.

To apply the technique to gas chromatography, easy repetition of highly reproducible chromatograms is required. A high-precision gas chromatograph, such as the one described by Oberholtzer and Rogers (2) provides for excellent control of column temperature and flow rate, and it incorporates digital control of sample introduction and digital data acquisition. As a result, retention volumes for well behaved chromatographic systems have been reproduced to better than $\pm 0.02\%$.

From the practical viewpoint, ensemble averaging shows obvious promise for analyses involving very low concentrations of components in samples. However, it should have applications in basic chromatographic studies as well. For example, the behavior of the capacity ratio is rather poorly understood for very dilute samples.

Experimental

Apparatus. The chromatograph used in this study was essentially that described earlier (3). In the present experiments, the signal from the flame-ionization detector was such that the electrometer could be operated at 3×10^{-13} amp full scale. The noise on the signal was approximately 5×10^{-14} amp.

A Seiscor sampling valve (Seismograph Service Corp., Tulsa, Okla.) having a 25 μ l sample loop was used for injection of the samples onto the chromatographic column. The 50-cm x 0.32-cm id stainless steel column was packed with 5% SE-30 on Chromosorb G and was operated at 65.84°C.

The carrier gas was high purity helium (Airco High Purity grade) which had been passed through a 4A molecular-sieve trap. The trap had been freshly conditioned overnight at 400°C while being backflushed with helium at 1 to 2 ml/min.

Data reduction and manipulation were carried out on a Hewlett Packard 2116A computer with an attached magnetic tape deck.

Sample Preparation. A binary gas mixture, which consisted of methane (Matheson Co., C.P. grade) in helium was prepared according to the method of Guiochon et. al. (4). The suggestion of these authors for replacement of several absolute pressure measurements by differential measurements was implemented in our system so as to prepare a concentration of 540 ppm of methane in the helium stream. A 0.65 ml sample of the binary gas mixture was injected into an exponential

dilution flask (5) by means of a Carle Model 2014 Sampling Valve equipped with a Model 2050 Pneumatic Actuator (Carle Instruments, Inc., Anaheim, Calif.). The helium flow through the exponential dilution flask was 137.5 ml/min. Assuming that complete mixing occurred instantaneously, the initial concentration of methane in the flask was 2.90 ppm. The effluent from the dilution flask was sampled at 30-sec intervals from 120 to 540 sec after introduction of the sample into the flask. Over that span of time intervals, the concentration range of the effluent from the exponential dilution flask extended from 300 ppb to 0.100 ppb. The amount of methane actually injected onto the column from the effluent stream ranged from 5.10×10^{-11} to 1.70×10^{-14} g.

Data Handling. The digital data obtained from the chromatograph were transferred from paper tape to magnetic tape using a Hewlett-Packard 2116A computer. The ensembles were then established by sorting and retrieving from the magnetic tape the appropriate chromatogram in each dilution series.

Peak detection was accomplished by manually selecting points which corresponded to the beginning and end of the peak. The baseline for the peak was determined by carrying out a linear least-squares fit of the 75 points prior to the peak and the 75 points following the end of the peak. The data points over the peak were then corrected for the baseline and the area of the peak was ascertained. The

mean retention times were calculated by first-moment analysis of the peak. Retention times corresponding to the peak maxima were also determined by fitting a second-order equation over the top of the peak by the Crout data-reduction technique (6). Appropriate first-derivative calculations were then carried out to obtain the value for the peak maximum.

A weighted nine-point smooth, after the method of Savitsky and Golay (7) was carried out on the ensembles of data. The peak parameters described above were also calculated for the smoothed data sets.

Results

To determine the random nature of the noise in the chromatographic system, a series of preliminary experiments were carried out in which pure helium was injected onto the column. In those studies, a pressure spike was observed upon interruption of the flow through the column while sampling. The chromatographic baseline was found to stabilize again within three seconds after the pressure surge. Following that initial deviation from random noise behavior, no other divergence was observed in an ensemble consisting of 200 baseline sets.

Typical behavior of an ensemble as a function of the number of chromatograms in the average is shown in Figures 1-3. Figure 1 shows a single chromatogram from Series 6 which corresponded to a concentration of 18 ppb in the sample stream.

Figure 2 shows the display of an ensemble from Series 6 where 10 chromatograms were included in the average. Theoretically, this ensemble should correspond to an improvement in the signal-to-noise ratio of a little more than three. The nature of the ensemble, when 60 different chromatograms were included in the average, is shown in Figure 3. The enhancement of the signal-to-noise ratio in this case should be a little less than eight. Note that the peak mean and the peak maximum appeared to remain the same as the number of chromatograms included in the average was increased.

One would expect the peak area to be a linear function of the number of ensembles. However, as shown in Figure 4, a smooth curve was obtained in this set of runs for Series 2 and 4 which are representative of the results for concentrations between 30 and 300 ppb. However, a steady rise in the barometric pressure totaling 8-mm of mercury had been observed during the 20-hour period in which the data were taken. After correction of the data for the decrease in response of the detector with pressure (8), a nearly linear relationship resulted.

Another type of deviation is illustrated by Series 6 and 8, which are representative of results obtained at concentrations ranging from 30 to 0.1 ppb. For intermediate numbers of ensembles, the plots approached linearity and extrapolations of the linear portions passed near the origin. For the smaller ensembles, deviations from linearity became

quite marked when the signal-to-noise ratio fell below two. Those deviations were either larger or smaller than the normal extrapolated curve as would be expected for a relatively large contribution from random noise.

Another factor that led to a deviation was encountered at the lower concentrations for larger ensembles. In these cases, the areas were smaller than expected. By examining all of the ensembles, a second very small peak was found to appear after the methane peak. Because the extraneous peak remained the same size regardless of methane concentration, it was suspected of being a trace contaminant in the helium used for sample dilution. That suspicion was confirmed when the peak did not appear in a series of runs for which a different tank of helium was used. For those ensembles in which the impurity peak did appear following the methane peak, a higher-than-normal value for the baseline was obtained. Such an impurity, therefore, resulted in a loss in the area of the methane peak.

Figure 5 shows the areas of the averaged chromatograms as a function of the concentration of methane at various sampling times. The relationships appear to be linear for the larger concentrations of methane, but they display discrepancies at the lower concentrations near 5 ppb. Again, it was found that the discrepancies appeared when the signal-to-noise ratio was less than two. Least-squares fits in the region above the noted discrepancies yielded lines having standard

deviations of 7% and 12% for the ensembles of 60 and 10 chromatograms, respectively.

To determine the effect of smoothing, the data for all of the ensembles were smoothed and the appropriate peak parameters were determined. As a typical example, Figure 6 is the smooth of the data shown in Figure 3. Figure 7 shows that the peak areas from smoothed and non-smoothed ensembles lay on the same curve. Therefore the smoothed ensembles were more pleasing to the eye, but no advantage was gained by determining the peak areas from smoothed data when using the present method for defining the peak.

Table I shows the effect of sample size on the retention time for all ensembles of 65 or more runs where the signal-to-noise ratio was greater than two. In those data, there was a regular trend to slightly longer retention times as the methane samples became smaller. Those differences corresponding to a 5% total change in capacity ratio were rather small, and may, therefore, be meaningless. On the other hand, they may be real and may be the result of sorption effects occurring in the column. Additional work is underway to determine the validity of the change.

Discussion

Using a high-precision gas chromatographic system, ensemble-averaging techniques have been shown to be quantitatively successful when the signal-to-noise ratio of the final data array was greater than two. This lower limit occurred at a

methane concentration of 5.8 ppb for a 60 chromatogram ensemble. In view of the fact that methane was retained only very slightly under the conditions studied, the corresponding concentration limits for more strongly retained substances would be higher -- for the same level of detector sensitivity. Nevertheless, a significantly lower level of detection for any substance should be attainable using ensembles as compared with the usual approaches.

For example, trace components are usually concentrated prior to an analysis by cooling the forepart of the column or by cryogenic trapping just in front of the column. However, the quantitative aspect of a trapping technique often leaves much to be desired. On the other hand, in addition to eliminating the preconcentration step, ensemble averaging also improves the signal-to-noise ratio for an equivalent-size sample. This has been well demonstrated in spectroscopic studies, such as the one by Allen and Johnson (9), who showed that enhancement of the signal-to-noise ratio could be obtained by ensemble averaging the data for a dilute solution rather than making a single determination of a larger sample which gave approximately the same overall signal. Therefore, the application of ensemble averaging techniques to trace analysis should often be more desirable than preconcentration for one-shot determinations.

Ensemble averaging should also be an ideal method for continuous monitoring of trace constituents. One could use a fixed-size ensemble and continually update the ensemble by adding

a new chromatogram to the ensemble while subtracting the oldest. Such an approach would cut down the time-lag between actual changes and their detection, while also providing much more data about those changes. Studies of such systems involving multi-component samples are underway and have shown promise (10).

As shown in the present study, one of the limiting factors will be the effect of fluctuations in the atmospheric pressure on the response of the flame-ionization detector in any averaging process that extends over a long period of time. However, there are two practical solutions to this problem. One possible solution would be to enclose the detector in a constant-pressure chamber (11). An alternative solution, which would be especially easy to adopt if the gas chromatograph were connected to an on-line digital computer, would be to read the pressure before each run and correct the detector response to that for a standard pressure.

It is important to note that an extensive investigation of derivative peak-sensing was carried out at the beginning of the present study but was then abandoned. A large amount of spurious triggering was observed with noisy data when thresholded-derivative peak-sensing was used. When a continuity criterion was added, which required that an appropriate condition remain true for a specific length of time before acceptance of that criterion, the end of the peak could not be established. Thus, derivative peak-sensing is not suitable for peak detection in very noisy chromatograms.

A second approach to peak detection, the method used in this study, involved integrating over a sector of the averaged chromatogram after establishment of the baseline from points on both sides of the integration sector. That sector was easily determined by visually observing the location of the peak in the larger ensembles. Although this method was satisfactory for most of our work, there are inherent problems associated with it. One problem is the inability to cope with unresolved peaks. A second problem, which was encountered in the present study, arises when an unexpected peak is present in the sections of the chromatogram used for the determination of the baseline. However, the latter problem could be minimized by permitting the operator to interact with the displayed data through the computer.

Although the emphasis of the present study was on peak area, it was noted that the retention time of methane showed a small but significant trend to longer retention times for smaller samples. One cause for such behavior might be a change in the activity coefficient. However, that phenomenon seems unlikely, at least in the present situation, due to the fact that the most concentrated sample was already quite dilute. A second possibility would be the increased contribution of sorption relative to liquid partitioning. In many instances, sorption is the energetically favored interaction, but due to the small surface area of the liquid with respect to its volume (12, 13)

the contribution of sorption has little overall effect. However, with very small samples, sorption effects, such as those occurring on the gas-liquid-interface, on the solid support, and on the column wall, become relatively more important. For that reason, chromatographic studies are underway to investigate adsorption effects that are encountered when very dilute samples are used with common stationary phases.

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TABLE I. EFFECT OF CONCENTRATION ON RETENTION TIME FOR METHANE

Series Number	Concentration, ppb	Retention Time, Sec	
		Mean	Maximum
1	304	11.95	11.87
2	174	11.98	11.89
3	97	11.97	11.87
4	56	12.00	11.90
5	32	11.99	11.90
6	18	12.08	11.93
7	10	12.06	12.10
8	5.8	12.14	12.33

Figures

1. Chromatogram for 18 ppb methane in the sample stream.
2. Ten-chromatogram ensemble for 18 ppb methane.
3. Sixty-chromatogram ensemble for 18 ppb methane.
4. Increasing numbers of chromatograms in the ensembles.
5. Ensemble averaged peak area as a function of methane concentration.
6. Nine-point smooth of the sixty-chromatogram ensemble for 18 ppb methane.
7. Comparison of peak areas from smoothed and non-smoothed ensembles.













