COO-1222-43

Gas Chromatographic Studies of Rapid Repeated Injections of Samples Into a Column

by

Donald Macnaughtan, Jr., and L. B. Rogers

Department of Chemistry

Purdue University

Lafayette, Indiana 47907

DISCLAIMER

Brief

More frequent analyses from a single column and, in some cases, virtual deconvolution of overlapped peaks and reduction of tailing have been demonstrated.

Abstract

Computer-simulation and laboratory studies have been made of quantitative analyses obtained by overlapping of two or more chromatograms in a single column. Using a high-precision chromatograph, the compromises between extent of overlap, which increased as the sample-injection interval decreased, and the resulting accuracy and precision have been examined. Overall, rapid repeated injections permit a more nearly continuous analysis of a sample stream using a single chromatographic column. One potentially valuable special case has been discovered which involves summing the peaks of more strongly held components. In a second important case, a virtual deconvolution of overlapped peaks can be accomplished. In a third case, excessive peak-tailing has been shown to disappear under appropriate conditions.

Rapid Repeated Injections of Samples into a Gas-Chromatographic Column

In an effort to process more samples in a given time using only a single gas chromatograph, several ideas have been tried. First, Reilley, Hildebrand and Ashley (1) suggested a periodic introduction of samples into a column and subsequent Fourier Analysis of the waveform appearing at the detector. Second, Hiratsuka and Ichikawa (2) continuously injected a sample but changed the amount in a sinusoidal fashion. They used several detectors along the column so as to measure the phase-shift and amplitude-change from the original wave. By using a set of simultaneous equations, they were able to calculate the mole fraction of each component. Third, Obst (3) injected a sample periodically and applied a phase modulation treatment to the waveform that appeared at a single detector.

Recently, Power (4) and Murdock (5) used a simpler, but more limited, technique of repeated injections. In both cases, one of three components was held up for a long period of time compared to the first two. The conditions that resulted in long retention for the last component were necessary in order to separate completely the first two components. Their approach was simply to fill the interval between the rapidly eluting components and the strongly held one. Both investigators stopped injections at the time after which they would have had severe overlapping of components, thereby allowing elution of the strongly retained third component from that series of samples before proceeding with another series. Both

investigators reported a significant saving of analysis time.

The purpose of the present investigation was not only to produce more results in less time, but also to do so in a more nearly continuous manner. For example, by judicious choice of the time interval between injections, Power's analyses would not have had to be interupted in order to clear the column periodically. As will be shown later, even if there had been no interest in the strongly retained component, repeated periodic injections would save time compared to backflushing or allowing the most strongly held component to elute.

To apply any of the above methods, it is necessary to determine the relationship between the time between successive analyses and the attainable accuracy and precision. For example, no accuracy is lost by overlapping of samples by repeated injections if the individual peaks are still completely resolved. However, as the injection interval is made shorter and peaks begin to overlap, the accuracy and precision of the analyses will decrease. The question then becomes one of how closely one can approach a continuous analysis and still obtain results of prescribed accuracy and precision.

Assuming that one has a high-precision chromatographic system, the present approach offers advantages over the alternatives discussed above. First, it not only uses a single column and a single detector, but also a simple approach toward calibration. Second, it permits measurements to be made at a few selected times rather than over all of the data points. Third, treatment of a system which involves severe tailing is simplified because the overlap of samples

will deactivate the most energetic adsorption sites to a much greater extent than usual. As expected, one has to examine the effects of the components on one another and of peak distortion from instrumental as well as from physico-chemical sources.

Experimental

Reagents. The hydrocarbon samples, n-pentane, n-hexane, and n-heptane, were J. T. Baker "Analyzed Reagent" G.C. - Spectro quality. The helium was Airco 99.99% pure that had been passed through a 4A molecular sieve. The column packings were Davison 40-80 mesh Chromosorb G impregnated with SE-30, and silica gel.

Procedures. A 4% w/w SE-30 column was prepared by dissolving the SE-30 in chloroform and coating it on the Chromasorb G using a rotary evaporator to remove the solvent. The coated support was packed in a 100 cm x 0.32 cm o.d. copper tube by vibration. Another column, containing silica gel, was packed in the same way using a 50.0 cm length of the same tubing. A second coiled silica column was 50 cm x 0.64 cm o.d. All of the columns were formed into 10.0 cm. i.d. coils.

Liquid samples were injected using a 5 µl Tracor valve. Gas samples were produced in the following manner. A stream of helium was passed in succession through two gas saturators which contained the liquid hydrocarbons. A fiber-glass plug was located in the exit port of the last tube to trap any droplets in the stream. The saturators and filter were completely immersed in a dry-ice-methanol bath ($\sim -78^{\circ}C$) contained in a Dewar flask. The saturated

helium stream was allowed to warm to room temperature and then passed through a 25 µl Seiscor sampling valve. Under those conditions, one injection corresponded to about 0.5 ng of n-hexane. Using a chromatographic system described previously (6), an injection sequence was obtained by using a digital counter to select a time interval. Each time the preset count was reached, the sampling valve was actuated.

Simulation studies were performed on a Hewlett-Packard 2116A computer. The simulations were produced using a BASIC program called SIM, which permitted cathode-ray oscilloscope displays of Gaussian curves of desired height, width, skew, and position. Replicates of the curve(s) could be located at desired equal intervals along a time exis, and the sum of the heights of all of the peaks could be shown at each point along that axis. The running sum was the simulated chromatogram which was displayed on a Tektronix 611 oscilloscope and photographed.

The same computer was also used for reading the experimental data from the punched paper tape and for calculations.

Results

Simulation Studies. First, repeated injections of a one-component sample were examined using the Gaussian peak form. A chromatogram similar to Figure 1a was produced when the injection interval allowed each peak to be separated from the others. As expected, those measurements yielded results having accuracies and precisions comparable to those for a single injection. Figure

1b shows a simulated chromatogram for the same sample injected at a shorter interval. Consequently, the peaks overlapped somewhat and some accuracy and precision was lost. The height of each peak was still known accurately because the neighboring peaks did not overlap enough to affect the height appreciably, but the valleys between the peaks were beginning to fill in,

As the injection interval was decreased further, the valleys filled in faster than the peaks increased. Figure 1c shows the signal from an injection interval short enough so that there was only a somewhat wavy plateau which was higher than a single peak. Further decreases in the injection interval had the major effect of raising the plateau and causing it to be more nearly flat. This last example has now approached quite closely the limit of continuous injection.

By calibrating the system, the height of the plateau can be related to the amount of sample in each injection. Figure 2 describes the maximum height of the overlapped peaks relative to a single Gaussian peak vs the injection interval expressed in standard-deviation units of the Gaussian peak. In going from complete separation of the peaks down to about 3 standard-deviation units, the peak height increased less than 2%. Then, the relative height changed with increasing rapidity, but it could still be related quantitatively to the concentration (or amount) of sample.

The flatness of the plateau can be reported as a percentage by taking the difference between the average values for the maxima and minima, dividing by the average value for the maxima,

and multiplying by one hundred. When the percentage variation in the plateau produced from a Gaussian peak was plotted against the injection interval, the results shown by the solid lines in Figure 2 were obtained.

Simulations were also done using a skewed Gaussian model generated by means of the following equation (7).

$$\underline{Y}_{\underline{x}} = \underline{H} \exp\left[-(\underline{x}-\underline{c})^2 / 2(\underline{w} + \underline{s}(\underline{x}-\underline{c}))^2\right].$$

where $\underline{H}$ is the height of the peak, $\underline{x}$ is the position along the abscissa, $\underline{c}$ is the location of the peak center on the abscissa, $\underline{Y}_{\underline{x}}$ is the ordinate value for a given $\underline{x}$ value, $\underline{w}$ is the standard deviation of the basic peak, and $\underline{s}$ is the skew factor, which was held equal to zero for all values of $\underline{x}$ before the peak center. That input produced the normal Gaussian form on the leading edge, while on the tailing edge, it could be used to progressively increase the peak tailing. The dotted lines in Figure 2 show that this particular form of peak skew did not produce as flat a plateau as the pure Gaussian form at injection intervals less than 3 standard deviation units. In addition, each wave in the plateau was skewed. These results were confirmed by a simulation study using data taken from a real chromatogram for a skewed peak of n-heptane on a silica column. The percent variation was essentially the same as that of the artificially skewed Gaussian. This phenomenon will find application later in this paper.

Two-component systems were also considered so that all the baseline in a chromatogram would be utilized to produce meaningful data. For any two-component system, overlapping of the peaks is not the best solution for improving the rate of repeated analyses; the best choice is to shorten the column and not overlap the injections. This would do four things: cause the peaks to be narrower, permit the peak maxima to be closer together, shorten the time to complete a chromatogram, and, usually, simplify the data handling. Under optimum conditions, the injection interval would be equal to the sum of the base widths of the two peaks. Thus, as soon as the two peaks from the first injection had eluted, the second sample would begin to elute (Figure 3b). The individual chromatograms would not be overlapped.

However, the overlapping of two-component systems is useful to consider because one can often reduce, to the equivalent two-component system, problems which involve samples that contain three or more components. Then, one can often find a useful solution by applying the overlap method.

If two peaks are rather widely separated, one has the choice between speeding up analysis using that same column, by increasing the column temperature (or the flow rate of the gas) and lowering the column temperature so as to permit peaks from two chromatograms to be interspersed with little or no overlap of the individual peaks. To minimize the overlap of the peaks in the latter case, the injection interval must be $2\Delta T/n$ where n is an odd integer,

greater than 1, and ΔT is the time (distance) between the two peaks in a given chromatogram. In that way, the peaks will be prevented from directly superimposing on one another as a result of having a common multiplier of time. Figure 3a applies this concept to a two-component sample. If shorter intervals are used (greater n) the overlap is greater just as it would be if the two components in one sample were not as widely separated. For quantitative measurements, the maximum value of each peak can be calibrated using known mixtures. The response time of that system from the first injection until a "steady state" is reached will be equal to the elution time of the last component in the sample injection that follows the change.

As an alternative one can overlap not only the individual chromatograms but also the individual peaks. If, in a mixture, one peak is at least 2, preferably 2.5 times wider than the other, then the injection interval can be made such that the broader peak will overlap with itself forming a nearly flat signal as was described (Figure 2). However, the narrower peak will not overlap with itself to an appreciable extent. Under those circumstances, the narrower peak was found to sit upon the relatively flat plateau of the wider peak. The relative positions of the two peaks was unimportant, and, if they were unresolved, a virtual deconvolution was accomplished quite simply. Figure 4 shows a chromatogram of one such mixture (n-pentane and n-heptane) and the results to be expected from rapid repeated injections. In the homologous n-alkane series, the n and (n + 2) components will give the necessary ratio of widths. It should be possible to measure such a system using the maxima of the peaks

and the minima of the troughs. It should also be possible to analyze mixtures of the n and $(n + 1)$ members of a series, but with less reliability.

We shall now explore how the method of overlapping of chromatograms might prove to be useful for three or more components in a sample, where separation of any two of the components is difficult (4,5). In the first case, two components may be just barely resolved but the third component is widely separated from the first two (Figure 5). The baseline between peaks two and three can be utilized by considering peaks one and two as a single peak and applying the method of overlapping chromatograms. An injection interval can be selected that will form a plateau from the overlapped third peak, on which the resolved pair of peaks one and two will sit. However, the third peak must be greater than 2.5 times as wide as the first two together ($\underline{CD} \geq 2.5\ \underline{AB}$).

The second possibility is to employ a modification of the procedure employed by Powers (4) and Murdock (5). Depending upon the width of the separation between peaks two and three, different numbers of samples can be overlapped. For example, the separation distance, $\underline{BC}$, must be equal to, or greater than, the total base widths of the peaks, $\underline{AB} + \underline{CD}$, Figure 5. This is the case where n is equal to 3 and $\underline{AB}$ and $\underline{CD}$ are just able to fit into $\underline{BC}$. If $\underline{BC}$ is two or more times larger than $(\underline{AB} + \underline{CD})$, n can be larger, and shorter injection intervals can be used. In general, $(n - 1)/2$ is the number of total base-widths $(\underline{AB} + \underline{CD})$ which can fit into the $\underline{BC}$ interval. Thus,

$$\frac{(n - 1)}{2} = \frac{BC}{(AB + CD)} \qquad (1)$$

and, solving for n,

$$n = \frac{2\ BC}{(AB + CD)} + 1 \qquad (2)$$

Rounding off to the next lowest integer allows one to calculate the minimum injection interval.

Another possibility is that of deliberate overlapping of later peaks if only their sum is desired. This might be done in a situation where there are early peaks which are to be individually determined, and later broad ones for which only the sum is needed or, perhaps, they would otherwise be ignored or back-flushed. This type of problem arises in air-pollution analysis for sulfur dioxide or ammonia (8). To separate these from air, using Graphon, requires that a water peak appear long after the components of interest have eluted. The injection interval would be such that the later broad peaks sum up to a continuous baseline upon which the earlier sharp peaks would stand.

Laboratory Studies. The first experiment was aimed at determining how well the laboratory results produced from a chromatograph agreed with the results from the simulation study for a single component. A sample stream of helium, saturated at $-78^{\circ}C$ with n-hexane was sample periodically and introduced into the 0.32 cm silica column. The resulting peak had a standard deviation of 12.8 seconds at half height, was slightly skewed,

and had a tailing edge approximately 20% wider than the leading edge. The data were taken after three peaks of the series had appeared, and then no fewer than five measurements were made on each run. The data plotted as shown in Table I had a coefficient of variation of less than 2%. Figure 2 shows that they compared closely with the simulations using the Gaussian form (solid line). Since the hexane peak was slightly tailed, the results were not expected to be in such close agreement with the predictions based upon a Gaussian peak.

The closeness of agreement seemed to indicate that the peaks became more nearly Gaussian in shape due to coverage of the more active sites in the column. To show that this was indeed the case, a more severely skewed peak was obtained using 5 µl liquid samples of heptane injected onto the 0.64 cm o.d. silica column. Figure 5a shows the heptane peak produced. Upon going to repeated injections of the sample, this skewed peak formed a baseline like that derived from a Gaussian input.

To explore further the idea that deactivation of the most energetic sites was leading to a Gaussian peak, another experiment was run in which a constant amount of heptane was added to the helium carrier gas. Into that mixture, single samples of heptane were injected. As the continuous level of heptane was raised, the peak for the heptane samples became more nearly Gaussian, as shown in Figures 6b and 6c. The retention time also changed in the expected direction.

An experiment was also done on a mixture under conditions where the peaks in a single run would be badly tailed. Known amounts of n-pentane and n-heptane were used in mixtures to calibrate the system using the 0.64 cm o.d. silica column and a thermal conductivity detector. Approximately 5 µl samples were injected at 26-second intervals which corresponded to about 1.6 standard deviation units of the heptane peak. Each run involved about 25 injections. The ratio of peak widths was 2.7 so the pentane peaks were separated from one another by 4.4 standard deviation units of pentane at that injection interval. This gave a baseline (Figure 7b) upon which the narrower pentane peaks sat(Figure 7c). The percent variation of the baseline formed by the heptane was less than the noise (∿1%). Data taken on 8-10 peaks are shown in Figure 8 along with the least-squares lines. The greatest relative uncertainty in any run was 2.05 percent which corresponded to an absolute uncertainty in the mixture of $\pm$ 1.75% for pentane.

When the same experiment was repeated at an injection interval of 34 seconds (2.1 standard deviation units for heptane) a wavy signal of about 5.1% variation was obtained using pure heptane. Combined with the pentane signal, they produced a regular waveform of peaks and valleys. When samples of different compositions were done in random order, the reproducibility of the duplicates was 3.1% or better on a relative basis, or about $\pm$ 0.5% absolute in terms of pentante. Figure 9 shows the resulting calibration curve. Thus, even at the longer injection interval, which did not produce a flat plateau, the system could be calibrated to produce meaningful

results. However, the calibration curves were not linear. The reason for the nonlinearity is quite complex. It is a result of changes in the sample concentration which produce changes in the widths and retention times of the peaks as a result of changes in the percent coverage of the active sites in the column. Similar effects were observed previously in the one-component system (Figure 6). The curve for the difference did not go through zero because the heptane signal had a 5-percent variation at that interval and therefore there were peaks and troughs present even with pure heptane.

Discussion

The methods described above possess the advantage of saving a substantial amount of time without the need for added chromatographic equipment. Only one column is needed to process many samples quickly. For process-stream analysis, this system should give results at shorter intervals than single-sample methods, especially if one is interested in analyzing for only two or three components in a complex mixture. One would have to wait the same length of time to complete each analysis, but there would be more results per unit time, thus facilitating the observation of shorter-term changes in composition. The amount of data taken can be reduced by measuring only at a few selected times rather than continuously. The major requirement is the need for high precision in the sampling and injection system.

The analysis of the n and $(n + 2)$ system of a homologous system is possible because of the favorable peak-width ratio. It appears that this ratio will be favorable in most homologous series and

would thus be useful. Also, the n and $(n + 1)$ members of any series would lend themselves to the same type of analysis even though the ratio would be less favorable. Because of the lower ratio the narrower peak would overlap with itself to some extent. However, peaks and troughs would appear in the resulting chromatogram and would make possible an analysis after appropriate calibration.

The fact that the relative positions of the two peaks does not affect the "deconvoluting" effect should be emphasized. This fact allows one to do an analysis on a system without complete separation of the components as long as one component is at least 2.5 times wider than the other. Hence, the deliberate overlap of major-component peaks may permit the ready detection and determination of trace components that would otherwise be hidden by the major component. However, due to a small signal difference (trace component) in a large signal (major component), some sensitivity will be lost.

References

1. C. N. Reilley, G. P. Hildebrand and J. W. Ashley, Jr., Anal. Chem., 34, 1198 (1962).

2. S. Hiratsuka and A. Ichikawa, Bull. Chem. Soc. Japan, 40, 2303 (1967).

3. D. Obst, Journal of Chromatography, 32, 8 (1968).

4. J. L. Power, Pittsburg Conference on Anal. Chem. and Appl. Spectroscopy, Cleveland, Ohio, March 1-6, 1970.

5. H. R. Murdock, Jr., Anal. Chem., 42, 687 (1970).

6. J. E. Oberholtzer and L. B. Rogers, Anal. Chem., 41, 1234 (1969).

7. E. J. Levy and A. J. Martin, Pittsburg Conference on Anal. Chem. and Appl. Spectroscopy, Cleveland, Ohio, March 3-8, 1968.

8. A. DeCorcia, D. Fritz and F. Bruner, Anal. Chem. 42, 1500 (1970).

Supported in part by the U. S. Atomic Energy Commission under Contract AT(11-1)-1222.

Table I

Comparison of Real Chromatographic Data with Simulated Data for Rapid Repeated Injections of a One-Component Sample

Run	Injection Interval		Percent Variation		Relative Baseline Height	
Number	Seconds	Std. Dev.	Exp.	Simulation	Exp.	Simulation
3	72	5.00	90.0	91.2	1.10	1.00
4	48	3.33	55.0	50.5	1.12	1.01
5	36	2.50	16.6	16.2	1.13	1.08
19	32	2.50	17.9	16.2	1.17	1.08
21	25	1.95	4.1	2.1	1.38	1.30
23	19	1.48	0.7	0.2	1.78	1.70
25	16	1.25	0.6	<0.1	2.34	2.00

Figure 1. Chromatogram for repeated injections of a one-component sample at intervals expressed in standard-deviation units of the peak.

a - injection interval greater than 4 standard deviation units

b - injection interval of 2.5 standard deviation units

c - injection interval of 1.5 standard deviation units

Figure 2. Relative height and the percent variation vs the injection interval (standard deviation units) of a chromatogram obtained from rapid repeated injection of a one-component sample.

Solid lines - computer simulation using Gaussian form

Dotted lines - computer simulation using skewed Gaussian form

Points - experimental data

Figure 3. Chromatograms for a two-component mixture which compare the methods of overlap and no overlap. The injection number is shown.

Figure 4. Example of a virtual "deconvolution" by repeated injections of a two-component mixture. Solid line: - actual signal from chromatograph. Dotted line: - individual sample signals that form the chromatogram.

Figure 5. Three-component chromatogram illustrating unused baseline and the determination of the appropriate injection interval.

Figure 6. Chromatograms showing the changes in n-heptane peak parameters with the continuous concentration of n-heptane in the carrier gas.

A - no heptane in carrier

B - carrier saturated with n-heptane at 0 °C

C - carrier saturated with n-heptane at 20 °C

Figure 7. Example of typical results from a series of rapid repeated injections of a n-pentane - n-heptane mixture.

A. Chromatogram of a single injection

B. Result of an injection series with heptane only.

C. Result of the same injection series using the same sample as in A.

Figure 8. Calibration curve for the analysis of the n-pentane-n-heptane mixtures using rapid repeated injections of gas samples.

A. Trough minimum

B. Difference (C - A)

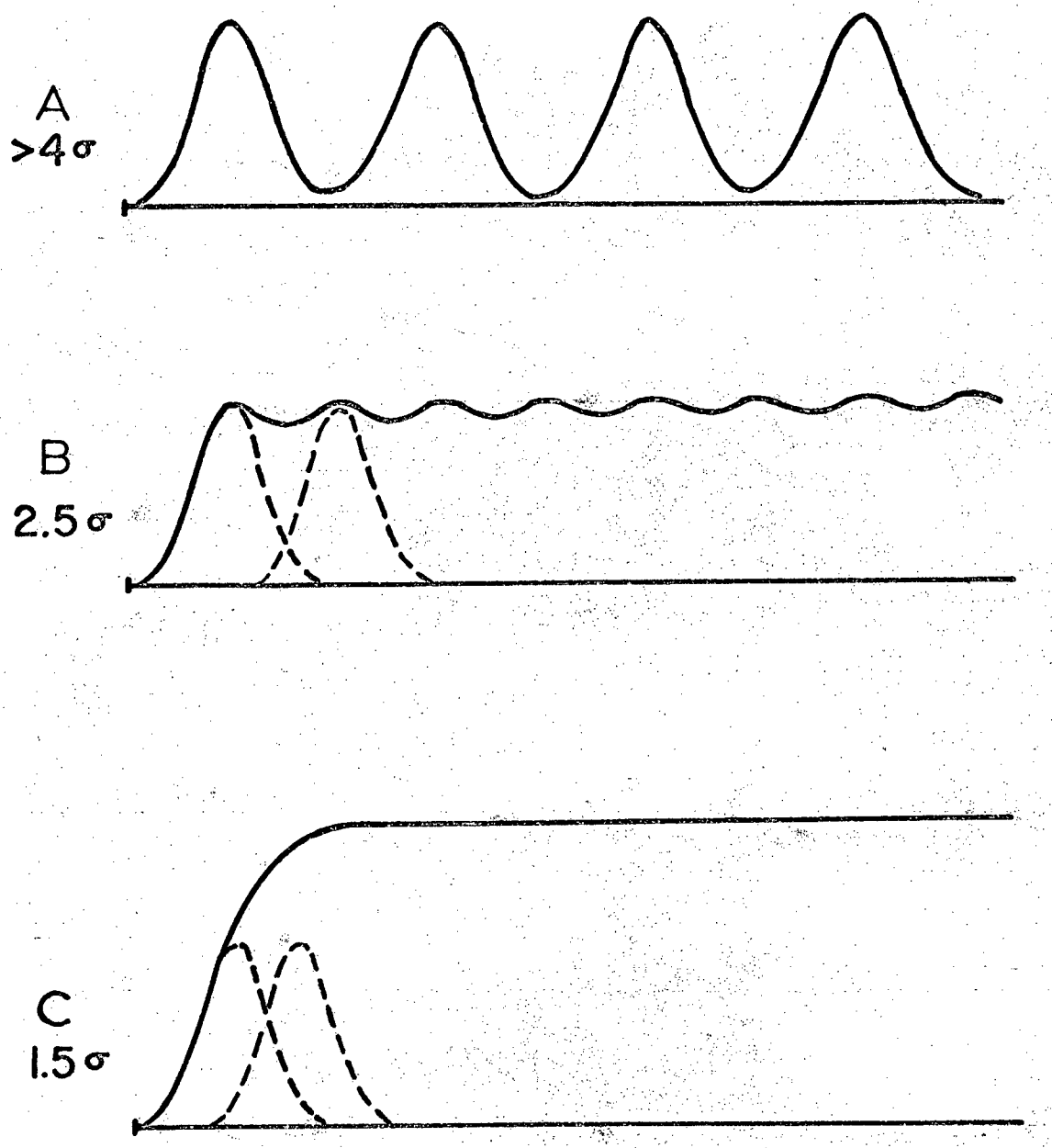
C. Peak maximum

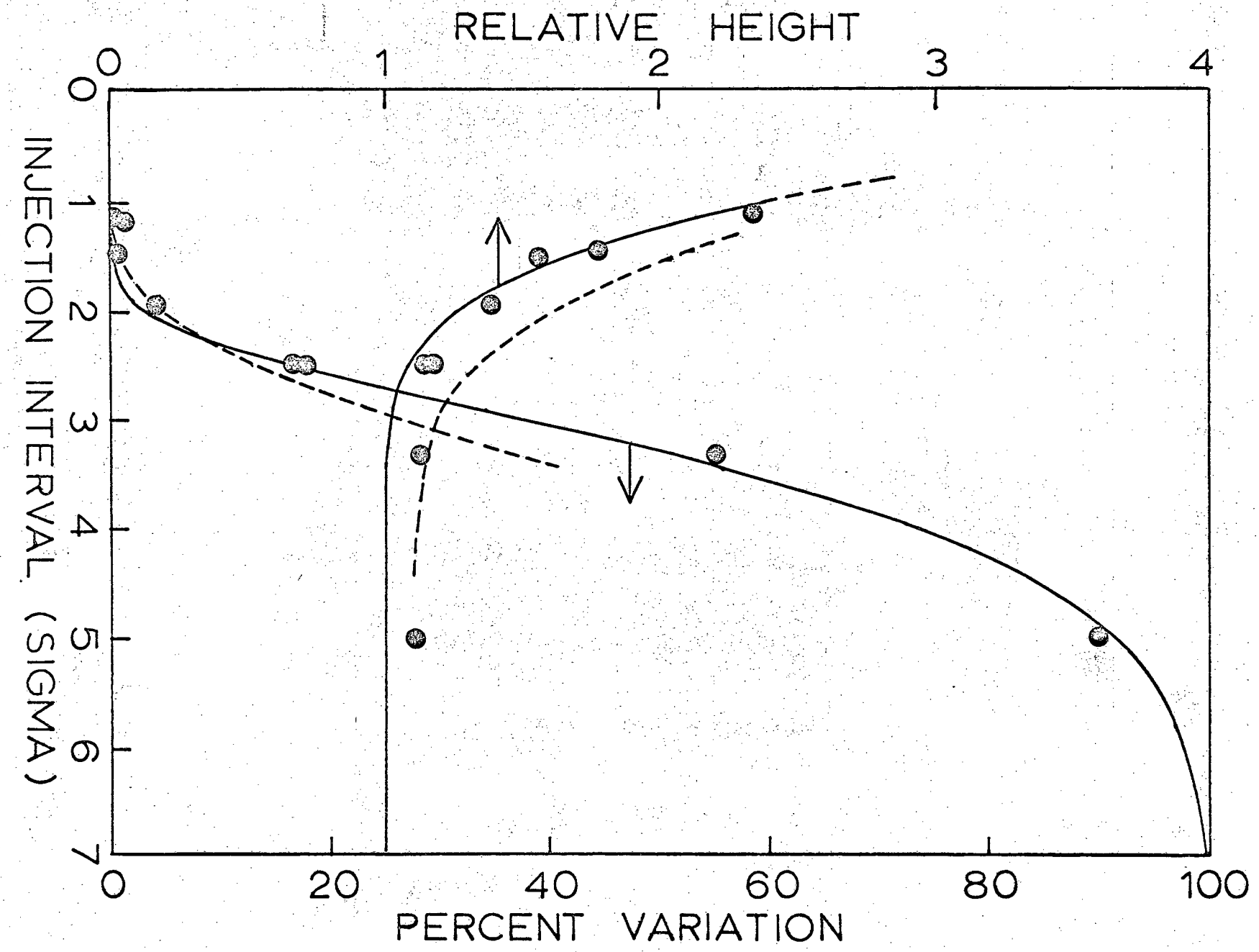
Figure 9. Calibration curve for the rapid repeated injections of liquid samples of a n-pentane-n-heptane mixture.

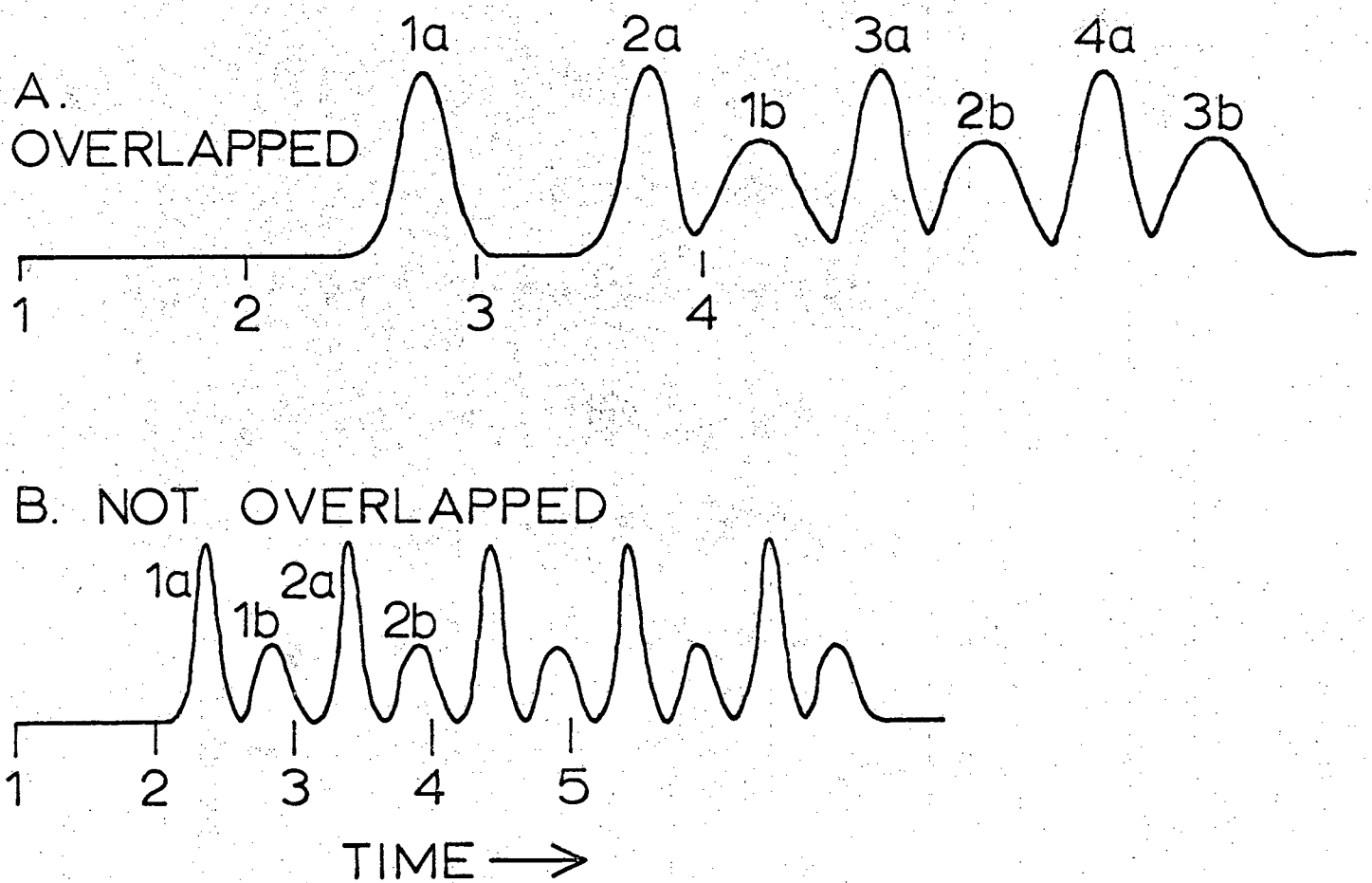
A. Trough minimum

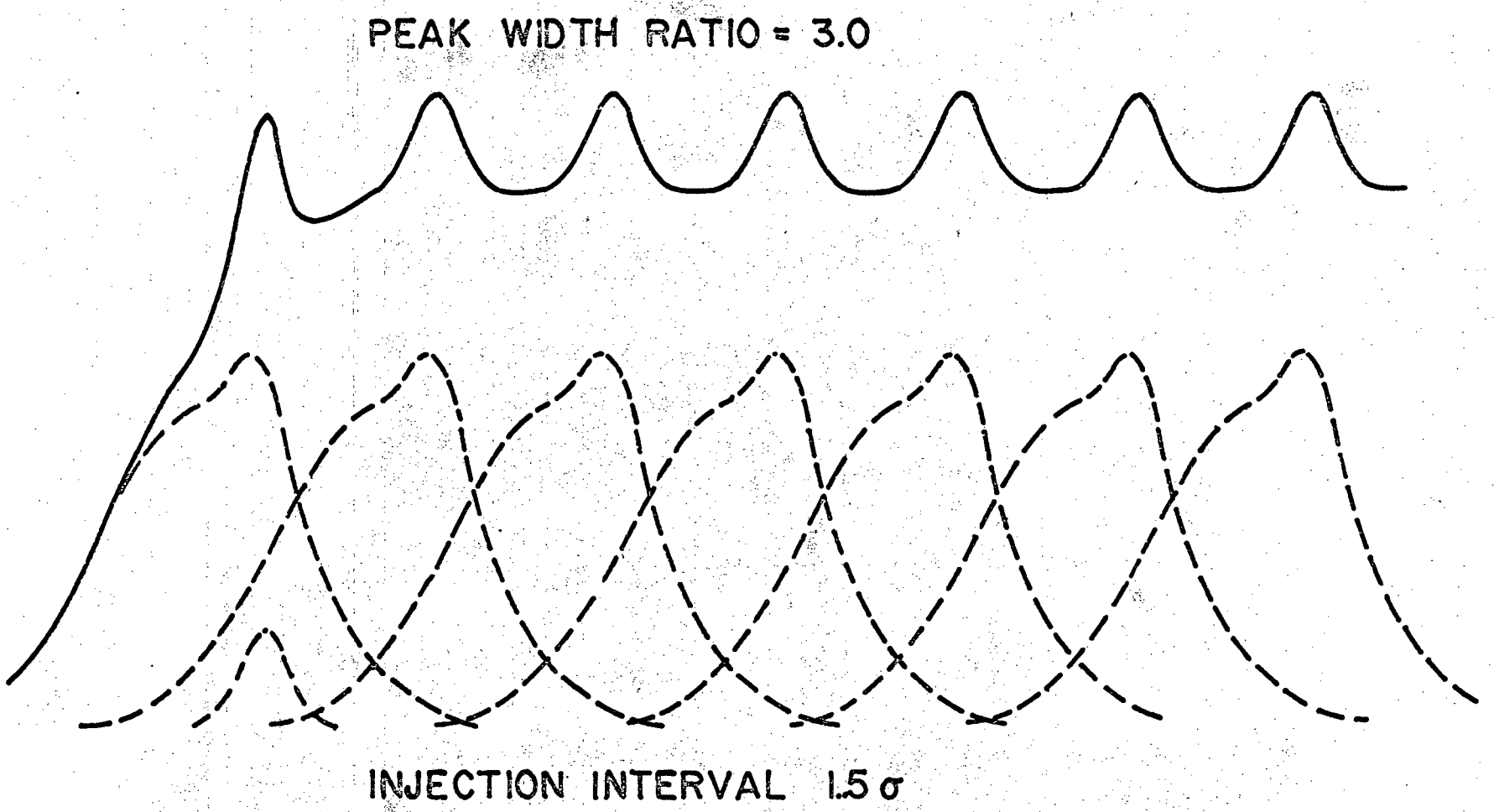
B. Difference (C - A)

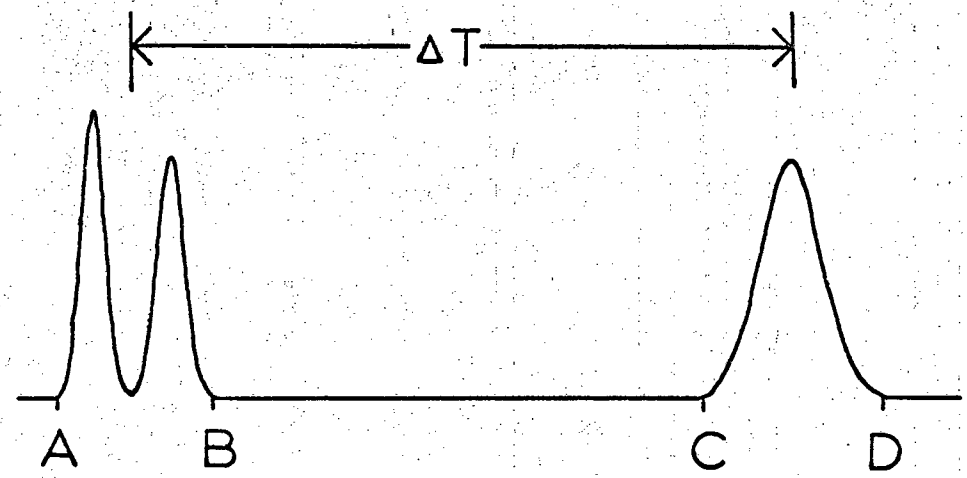
C. Peak maximum

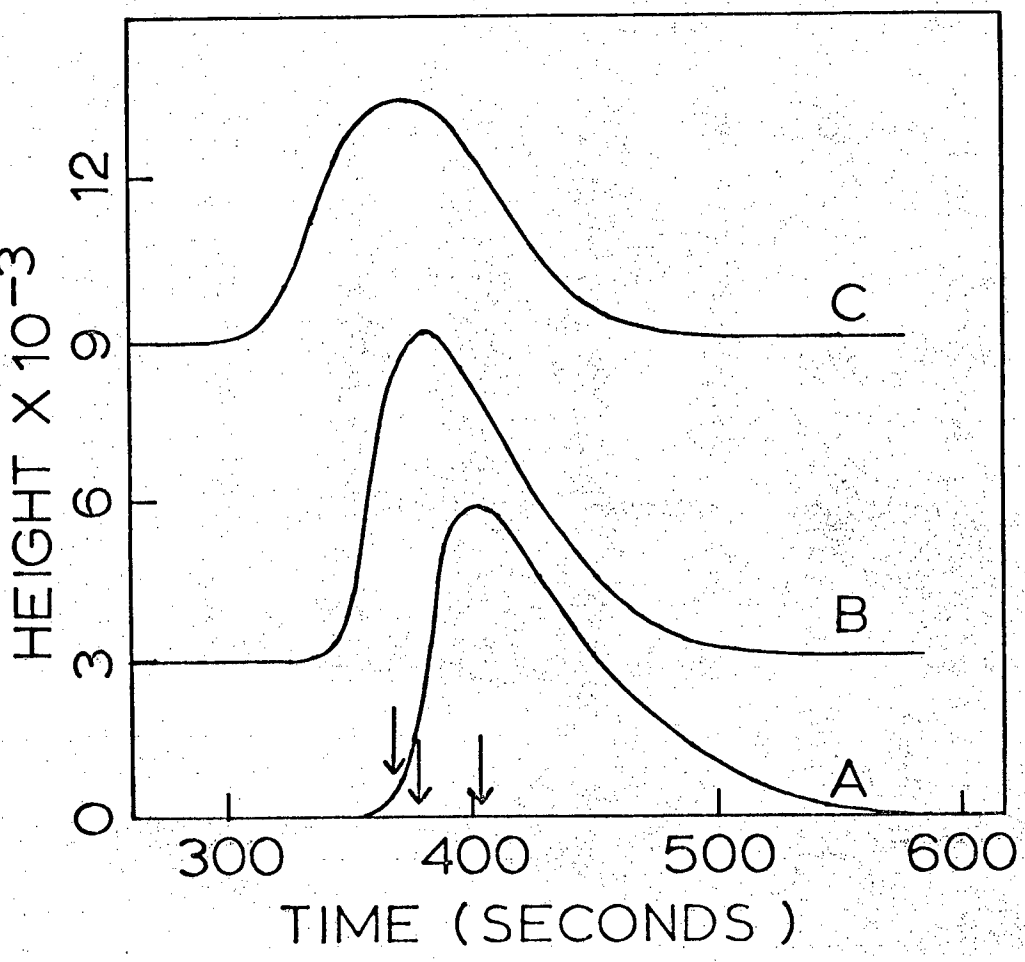

A
>4σ
B
2.5σ
C
1.5σ

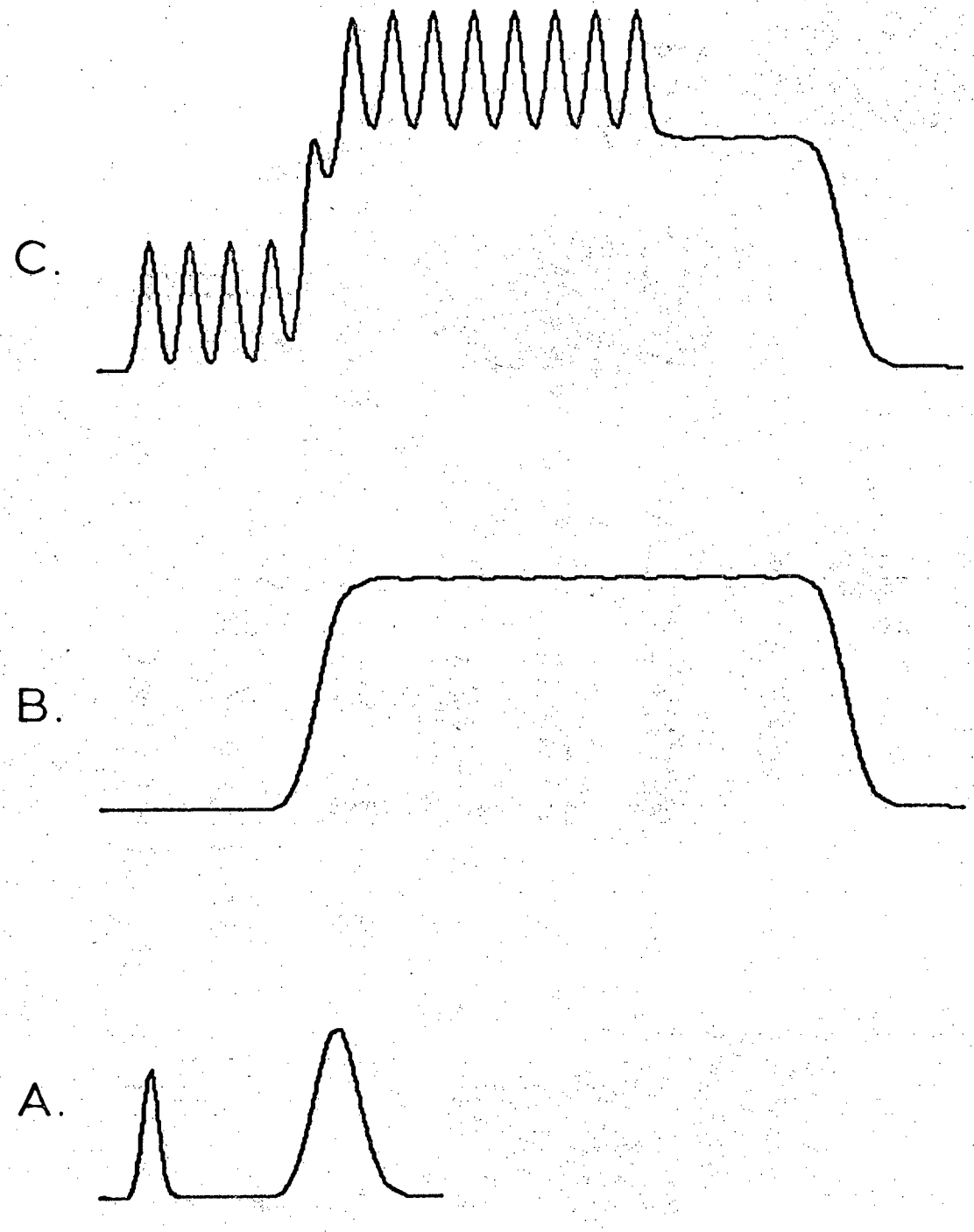

RELATIVE HEIGHT
0
1
2
3
4
INJECTION INTERVAL (SIGMA)
0
1
2
3
4
5
6
7
0
20
40
60
80
100
PERCENT VARIATION

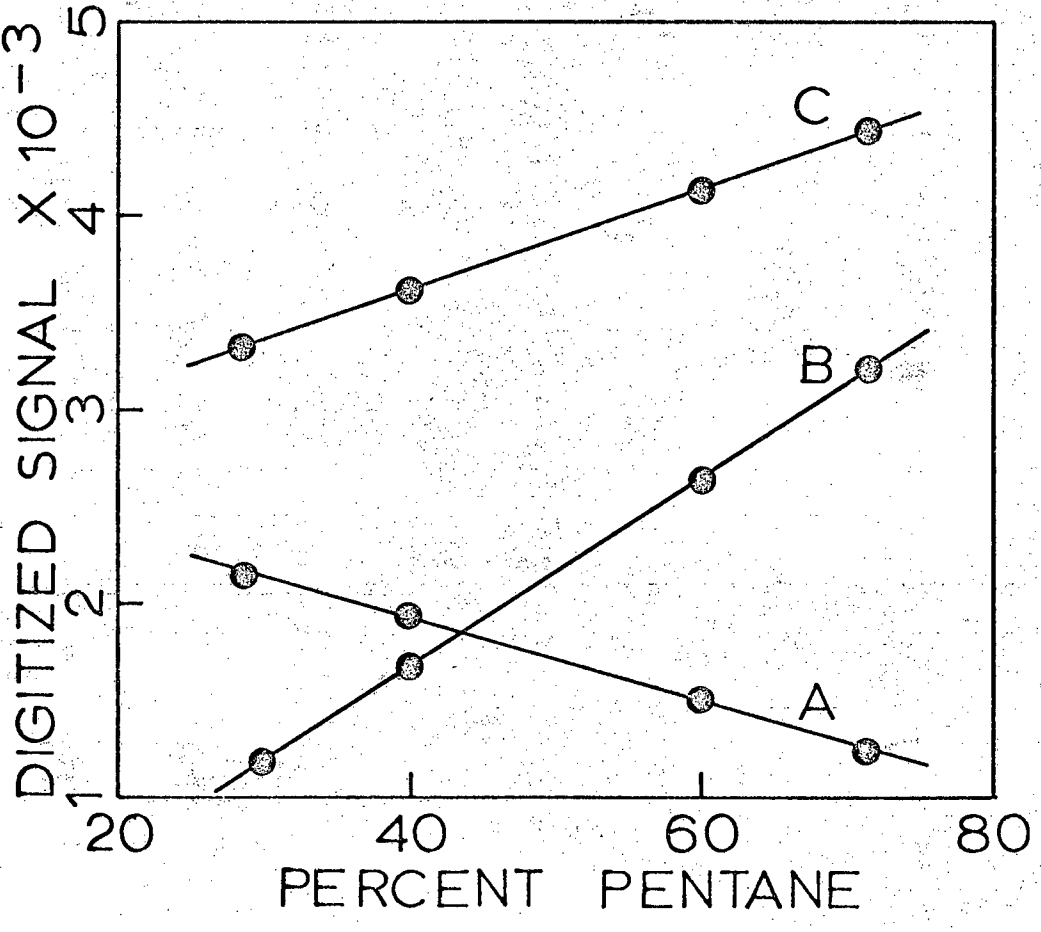

A.
OVERLAPPED
1a
2a
3a
4a
1b
2b
3b
1
2
3
4
B. NOT OVERLAPPED
1a
1b
2a
2b
1
2
3
4
5
TIME

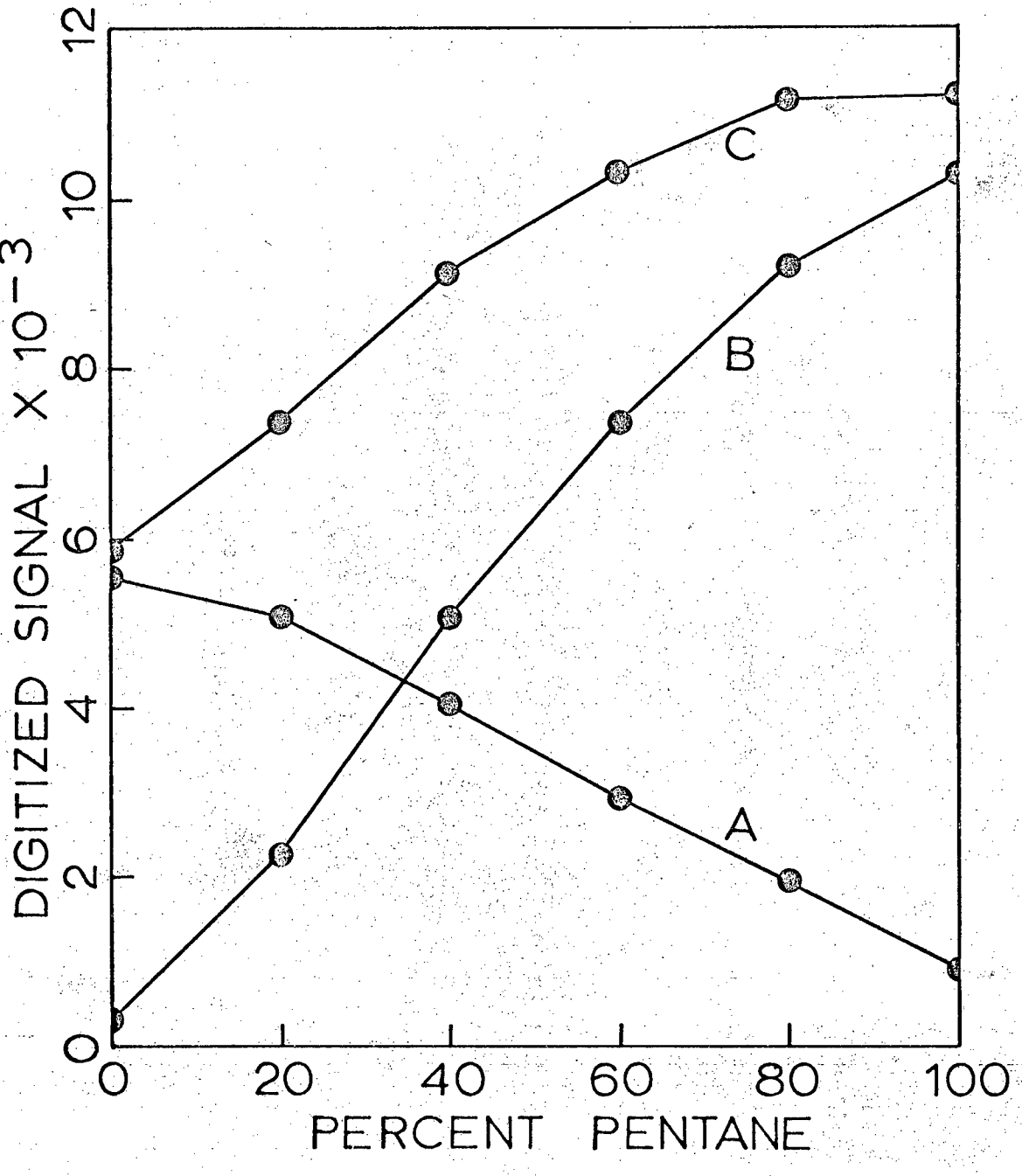

PEAK WIDTH RATIO = 3.0
INJECTION INTERVAL 1.5 σ


ΔT
A
B
C
D


HEIGHT X 10⁻³
0
3
6
9
12
300
400
500
600
TIME (SECONDS)
A
B
C


C.
B.
A.


DIGITIZED SIGNAL X 10−3
1
2
3
4
5
20
40
60
80
PERCENT PENTANE
A
B
C


DIGITIZED SIGNAL X 10−3
0
2
4
6
8
10
12
0
20
40
60
80
100
PERCENT PENTANE
A
B
C