Kinetic Parameters of Continuous Flow Analysis

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Unlike systems of batch analysis, continuous flow systems possess kinetic parameters. Associated with the steady state are such measurements as noise level and drift. This study reports on kinetic parameters associated with the transient state between the steady states including time required to change from base-line steady state to sample steady state and vice versa, characteristics of this change, time interval between samples, proportionality of sampling and washing time, fraction of steady state reached in any given sampling time, and interaction between samples. The transition between steady states has been found to obey first order kinetics to a good first approximation. This observation enables correlation of all of the above listed properties in quantitative fashion using new characteristic constants for continuous flow—the half-wash time ($W_{1/2}$) and the lag phase time ($L$). These parameters, well known in other contexts such as radioactivity, can be employed as "figures of merit" for any continuous flow system or component, can be utilized to calculate performance characteristics, and are useful in evaluating and optimizing over-all performance.

In a previous paper (1), 3 properties of continuous flow analytic systems were discussed which are different from those associated with batch analysis. These are drift, the effect of variation in sample depth, and interaction between samples. It is now common practice to monitor and control drift. Errors due to sample depth are minimized by sampling devices which provide rapid vertical motion of the sampling tube into and out of the sample such as the Sampler II (Technicon Instruments Corporation, Ardsley, N. Y.). Interaction is decreased by arranging that the inlet tube which picks up the sample is immersed in water whenever it is not in the sample (2).

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Nevertheless, significant interaction between samples can still be observed. Recent efforts have been directed at seeking the mechanism of its origin and the rules which govern it. In the process, it has been discovered that the transition between steady-state conditions in continuous flow analysis follows kinetics which are approximately first order with respect to concentration. This not only explains interaction, but also leads to a comprehensive mathematic basis for the continuous flow analysis.

To a good first approximation, the mathematic model presented here predicts the behavior of the output signal of continuous flow analyzers as a function of time. It has practical application in that it provides easily measured, simple, objective parameters for evaluation and comparison of continuous flow analytic methods and apparatus. Such parameters are needed because continuous flow analysis, unlike batch analysis, is so new that design and evaluation of methods is still subjective and, to some extent, an art. As a result, laboratories show marked difference, even in details of operation of the same method. For example, some people imply that there is special virtue in running a calibration curve from the sample of lowest concentration to that of the highest (3), while others defend the opposite practice. Nor do objective means exist for deciding the maximum or optimum rate (in determinations per hour) at which an analytic method should be run.

The parameters to be discussed provide simple measurable criteria by which one can evaluate quantitatively the effect of such factors as sequence of standards, sampling rate, proportion of sampling and wash time, interaction between samples, percentage of steady state reached in a given sampling time, and other similar considerations in continuous flow analysis. Because of the rules of first order kinetics, these factors can be described using one parameter, "half-wash time" (W₁/₂), with slight modifications due to the existence of a "lag-phase" (L). These 2 quantities may thus be employed as simple figures of merit which are useful as comparative, evaluative, and descriptive parameters for any continuous flow analytic method or apparatus.

**Apparatus and Reagents**

The apparatus and reagents employed in this work were extremely simple. A Heath strip chart recorder Model EUW-20A* was used, with chart speed of 5 inches per minute and full-scale pen excursion of one second. The output of the sample photovoltaic cell of a standard AutoAnalyzer was led to the input of the Heath recorder, but between the

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two, provision was made for momentarily short-circuiting the leads—
to ground the output signal and produce a time-marking pip. A stand-
ard AutoAnalyzer pump was used. The experimental manifold con-
sisted of only 1 tube which provided a continuous conduit from the
sample or water through the pump and N-type colorimeter to waste.
Sample introduction was accomplished manually by moving the inlet
end of this conduit at carefully timed intervals from water to sample
and vice versa.

Only one reagent was employed—a water-soluble red vegetable dye
ordinarily used for food coloration. From a stock solution of this dye,
a working solution with a measured absorbance (on the colorimeter) of
1.00 was made up. This solution was considered to have a relative con-
centration of 1.00 and further dilutions were made from this to give
solutions of lower relative concentration and absorbance. Corrections
were made for slight deviations from Beer's Law.

**Terminology**

Because terminology in this area of analytic chemistry is as yet
variable or nonexistent, it is necessary to introduce some terms and
abbreviations (Fig. 1). Figure 1A shows the strip-chart record tracing

![Diagram](image)

**Fig. 1.** Diagram for terminology. See text. $L$ and $L'$, low concentration sample; $H$, high
collection sample.
plotting the percentage transmission reading when one sample is aspirated for a considerable length of time. While the sample inlet is immersed in water (or in some chosen blank solution) the continuous flow instrument gives a reading of what is generally known as the "base-line steady state." In direct methods, this is generally set at 100% transmission or 0.000 absorbance. In inverted spectrophotometric methods, it is set at some chosen high value of absorbance or low percentage transmission. The inlet is placed in the sample at the beginning of the time period, designated "time in sample" (t_{in}). For a certain length of time, no effect is noted on the strip-chart record. The colored material takes time to reach the colorimeter and be measured. Let this be called the "delay time" (t_{d}). At the end of t_{d}, the measured absorbance suddenly starts to rise along a line called the "rise curve," and if t_{in} is sufficiently prolonged, a stable absorbance or %T reading will be obtained at the "Sample steady state." At the end of t_{in}, the inlet is removed from the sample and returned to water or a blank solution. This forms the beginning of the period of "time out of sample" (t_{out}). After the interval of time t_{s}, the initial effect of this change will be observed and the recorder absorbance will move along the "fall curve" until, if t_{out} is prolonged, the base-line steady state will again be reached. As shown in Fig. 1B (which omits t_{s} for clarity), at the end of t_{out}, the inlet is moved into another sample and another t_{in} begins. Obviously, the sum of t_{in} and t_{out} is the "time between successive samples" (t_{mn}). The units of t_{in}, t_{out}, and t_{mn} are, most conveniently, seconds, that of t_{s}, minutes. The "sample frequency" (f_s) in samples per hour is equal to \( \frac{3600}{t_{mn}} \).

In Fig. 1B and C, the situation is shown where t_{in} is so short that the strip-chart record does not reach the sample steady state. That percentage of the sample steady state concentration reached during the given t_{in} (%SS), may be expressed as the apparent concentration obtained at the peak, divided by the concentration of the sample steady state, and multiplied by 100 (Fig. 1C). One can, of course, observe exactly the same phenomenon and make precisely the same measurement for short values of t_{out} between prolonged values of t_{in}, where the base-line steady state replaces the sample steady state and vice versa. Thus, if t_{out} is sufficiently short, the strip-chart record of the apparent concentration will reach only a limited percentage of its full travel to the base-line steady state position. This phenomenon is seen as valleys between successive peaks.

"Interaction," I (t), can be measured by running a sample of low concentration, a sample of high concentration, and again a sample of low
concentration under the conditions of $t_{\text{int}}$, $t_{\text{out}}$, and $t_{\text{wa}}$ chosen for actual operation. When this is done, one can observe the concentrational error — due to interaction between the high sample and the second low sample — as a difference in the apparent concentration of the 2 low samples (points L and L' in Fig. 1C). “Percentage interaction” ($\%I$) is therefore given by the difference of the apparent concentration, divided by the high concentration which caused this difference, and multiplied by 100.

Method

Strip-chart records were made using a wide variety of $t_{\text{int}}$, $t_{\text{out}}$, $t_{\text{wa}}$, and sample concentrations. The characteristics of individual curves were examined directly from the strip-chart record — comparisons between different recorded curves were made by direct superimposition of the various strip charts over a light box. Complete objectivity with respect to superimposition of strip-chart records proved essential to conclusive experimentation, as did detection of instrumental malfunction so minor as to be ordinarily unobservable. These were achieved only after the utilization of timing pips was introduced. These pips, produced by momentarily grounding the recorder-colorimeter circuit at exactly the instant when any change was made (e.g., the movement of the sample inlet from water to sample or vice versa), enabled one to superimpose strip-chart records, using only the ruled horizontal lines of the strip-chart and the timing pips themselves to adjust the precise orientation of different curves. Other technics for superimposition which were tried proved to be subjective and often misleading.

Results

Determination of the Characteristics of Rise Curves

The results obtained on moving the sample inlet from water into each of 3 different concentrations of dye are given in Fig. 2. Figure 2A is a plot of percentage transmission vs. time and is a direct tracing of 3 separate experiments superimposed one on the other. Figure 2B is a plot of relative concentration obtained by plotting concentrations from several points on the curves of Fig. 2A. A number of different graphic expressions of Fig. 2B was attempted before the simple first order plot shown in Fig. 2C was tried. In reactions which show first order kinetics with respect to a given reagent, the rate of the reaction is proportional to the concentration of that reagent, as in Equation 1.

$$\frac{de}{dt} = -ke$$ (1)
Fig. 2. Rise curves. Dotted, dashed, and solid lines represent 3 different sample concentrations.

Fig. 3. Fall curves. Dotted, dashed, and solid lines represent same samples as in Fig. 2. C, open circles on dotted line are points from dotted line in Fig. 2C, replotted.
In the rise curve, however, the rate of change of concentration is shown by Fig. 2C to be proportional to the difference between the apparent concentration at any given time \( (C_t) \) and the final steady-state concentration, \( C_s \). This, in effect, means that the rate of change of the apparent concentration is proportional to the amount of the original water or blank solution left in the stream passing through the colorimeter at time \( t \). The linearity of the 3 curves in Fig. 2C, and the similarity in their slopes at different concentrations indicates that during the transient state in continuous flow analysis, the apparent concentration follows kinetics which are first order with respect to the difference between the apparent concentration and the steady-state concentration toward which it is proceeding, as in Equation 2.

\[
\frac{dc}{dt} = k(C_s - C_t)
\]

The slight curvature of the upper 2 curves in Fig. 2C, and the slight difference in the apparent slopes of these 3 lines may be attributed to the "toe" of the rise curve which occurs just at the end of the \( t_s \) at the 0-sec. mark on Fig. 2A. This reverse curvature is a lag phase, a phenomenon which is well known in the kinetics of chemical reactions, bacterial growth, etc., and which is discussed further below.

**Characteristics of Fall Curves**

The curves of Fig. 3A are reproductions of actual superimposed strip-start recordings of fall curves from 3 different relative concentrations. Figure 3A shows percentage transmission as a function of time; Fig. 3B shows relative concentration vs. time obtained by plotting concentrations from Fig. 3A. In Fig. 3C, the logarithms of these apparent concentrations are plotted vs. time. The data of Fig. 3C demonstrate that during the transient state of the fall curve in continuous flow analysis the change follows kinetics which are first order with respect to the apparent concentration itself, \( (C_t) \).

Thus, both rise and fall curves follow Equation 2, although in the latter case, \( C_s \) equals zero and drops out. The inherently identical nature of the transition in either direction is illustrated by the open circles of Fig. 3C which represent a replotting of the rise curve data for the sample of highest concentration. The points fall almost exactly on the fall curve for the same sample.

The fit of the 3 curves of Fig. 2C and 3C, and the similarity in their slopes show how well first order kinetics represents continuous flow analysis performance. The slopes of the straight lines give the rates of the transition reaction. It is convenient to borrow the "half-life" con-
cept from radioisotope usage and express these slopes and rates in terms of half-wash time—the time required to go from the apparent concentration represented by any point on the transition curve half the way (in concentration units) to the concentration at the steady state toward which the transition is heading.

**Effect of $t_{1in}$ on Rise Curves at Constant Concentration**

In order to determine the effect of various values of $t_{1in}$ on the rise curves for any one concentration, strip-chart records were obtained for one sample, at $C_{in} = 1.00$ relative concentration. The values of $t_{1in}$ shown in the data presented in Fig. 4 were 8, 10, 15, and $> 60$ sec. The rise curves from these strip-chart records were carefully synchronized in time by means of the timing pips and then retraced to form Fig. 4A. It is clear that for any given concentration, all the rise curves are merely parts of the one basic rise curve—which has been curtailed by removal of the sample inlet from the sample material before steady state has been reached. Thus, the initial slope of a rise curve, and the point it reaches after any given $t_{1in}$ are as much a measure of concentra-

![Fig. 4. Rise and fall curves at different values of $t_{1in}$.](image-url)
Identity of the Fall Curve

The strip-chart records from the experiment on the characteristics of fall curves were superimposed on each other by movement parallel to the time axis so as to superimpose the fall-curve portions of the peaks. As can be seen in Fig. 4B, the fall portions of all of these curves lie exactly on top of each other in spite of the fact that each comes from a different apparent concentration.

This is consistent with the results of the earlier experiment. Because they are all heading for the same steady state, namely the base line, all fall curves in a given system, for any concentration whatsoever, should have the same fundamental shape and should be superimposable upon each other by movement parallel to the time axis. Of course, these statements apply to rise curves and fall curves on strip-chart recordings of percentage transmission vs. time or concentration vs. time. If one plots rise or fall curves on a logarithmic scale vs. time, all transition curves for any concentration or time should be superimposable upon each other by movement parallel to the time axis, because all have the same \( W_{1/2} \).

Formation of the Sample Peak

One would guess that an approximation to the familiar sample peaks in continuous flow analysis should be formed from that part of the rise and fall curves permitted to form during a given \( t_n \). The data of Fig. 5 verify this. Figure 5 (Curve a) was obtained by taking a rise curve which reaches sample steady state at a relative concentration of 1.00
and superimposing on this strip-chart record that of a fall curve from this same concentration to base-line steady state. Superimposed upon these two is an actual peak obtained with this same sample solution, using a $t_{in}$ of 8 sec. The second part of Fig. 5 (Curves b and c) on the right side shows exactly the same procedure for a $t_{in}$ of 15 sec. but performed twice, 60 sec. apart.

Synchronization in Fig. 5 involves an additional consideration. Because the fall curve from the peak starts not at 1.00 but at an apparent concentration equal to the peak height, one can see that to achieve synchronization, one must place the timing pip of the fall curve after that of the rise curve by a number of seconds equal to $t_{in}$ minus the time required to go from $C_{as}$ to the apparent concentration at the peak height.

**Additivity of Overlapping Rise and Fall Curves**

The results of the above experiments imply that rise and fall curves might be expected to overlap when samples are aspirated one after the other. If this is correct, and if the phenomena described above in terms of half-wash time and lag phase are the only factors of major significance, then such overlapping should be additive in concentration.

In this experiment, 2 concentrations of dye and 1 of water were used to make 2 sets of curves. In each case, the complex curve produced from successive samples of the 2 different concentrations might be expected to be the concentrational sum at all times of the 2 simple curves, all carefully synchronized in time.

In Fig. 6A, the solid curve was obtained by placing the sample inlet successively in the high-concentration sample for a long period, in water for 20 sec. and in the low-concentration sample for a long period. The broken-line and dotted curves were traced from the fall curve for the high-concentration sample and the rise curve for the low-concentration sample, respectively, separated in time by 20 sec. and synchronized with the solid curve, all by means of the timing pips. Points a to e in Fig. 6 show actual concentrational values. Additivity, as illustrated at these points, is good.

In Fig. 6B, which is designed to mimic interaction, the solid curve was obtained by placing the sample inlet successively into the high-concentration sample for a long period, into water for 20 sec., into the low-concentration sample for 15 sec., and finally into water for a long period. One of the dotted curves was the fall curve for the high-concentration sample, the other was a peak obtained from a $t_{in}$ of 15 sec. using the low-concentration sample. These two were separated in time by 20 sec. and synchronized with the solid curve. Points a, f, and g, show concentrational values at one time, demonstrate additivity, and identify this
phenomenon as the significant origin of interaction. This phenomenon is also illustrated in Fig. 5.

**Direct Measurement of W_{1/2} and L**

One can plot \(-\log c\) vs. time from any strip-chart record which shows the complete transition from sample steady state to base-line steady state, obtain \(W_{1/2}\) from the slope of the straight portion of the line, and obtain \(L\) from the time at which the extrapolated straight line crosses the time axis curvature (Fig. 7).

![Fig. 6. Additivity of overlapping samples. Concentrations: point a, 0.26; b, 0.58; c, 0.88; d, 0.41; e, 0.80; f, 0.55; g, 0.83; a + b (0.84) should be compared with c; 2 \times d (0.82), with e; and a + f (0.81) with g.](image)

**Table 1. Measured Half-Wash Time (W_{1/2}) and Lag Phase (L)**

<table>
<thead>
<tr>
<th>Determination</th>
<th>(W_{1/2}) (sec.)</th>
<th>(L) (sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>10</td>
<td>24</td>
</tr>
<tr>
<td>Glucose</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td>Na</td>
<td>11</td>
<td>23</td>
</tr>
<tr>
<td>K</td>
<td>11</td>
<td>23</td>
</tr>
<tr>
<td>Cl</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>CO₂</td>
<td>13</td>
<td>17</td>
</tr>
<tr>
<td>TP</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td>Alb</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>Ca</td>
<td>9</td>
<td>32</td>
</tr>
<tr>
<td>Alk p'tase</td>
<td>21</td>
<td>8</td>
</tr>
<tr>
<td>SGOT</td>
<td>17</td>
<td>8</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>

* Data from Dr. A. Chasson (see acknowledgement).
This procedure was used to test the data obtained from the simple manifold described and also from one of the most complex continuous flow instruments in use, the SMA-12 hospital model (Technicon Instruments Corporation), to see if they fit this mathematic approach: both did. Table 1 gives values of \( W_{1/2} \) and \( L \) obtained from strip-chart records obtained on the SMA-12.

**Discussion**

In continuous flow analysis, the reaction being measured need not be complete for Beer's Law to be obeyed; but the degree of completeness should be independent of concentration because the reaction time, set by the flow rate in the manifold tubes, is constant. Therefore, to a first approximation at least, a study of the kinetics of transition such as this need not be concerned with the specific chemical reaction of the method in question but must concern itself only with the effects of longitudinal mixing. A simple dye solution as the sample material, and water as the base-line material, proved therefore to be as informative
as any chemical reaction one could choose—and much stabler, easier to handle, and simpler to interpret. The validity of this assumption is borne out by the agreement between the theory based on experiments with the simple dye solution and the results observed in complicated methodologies.

By adoption of the conceptual and mathematic treatment associated with half-life of radioisotopes, the expression of the interrelationships of various parameters of continuous flow analysis becomes simple and straight-forward with the $W_{1/2}$ as the key parameter. For quantitative calculation of these interrelationships, one need refer only to a generalized chart on semilogarithmic paper (Fig. 8) of the concentrational distance from steady state remaining at any given time (expressed as a percentage of the original concentrational distance) vs. the time itself (expressed in units of half wash time).

To determine the percentage of $C_n$ reached at any given $t_n$: divide the $t_n$ in seconds by the half-wash time in seconds, and read from the

**Fig. 8. Generalized first order chart.**
chart the percentage steady state which corresponds to that number of half-wash times. The procedure must be modified slightly where L is significant. In this case the number of half-wash times is taken as
\[
t_{\text{in}} - \frac{L}{W_{1/4}} \text{ instead of } \frac{t_{\text{in}}}{W_{1/4}}.
\]

An essential corollary of first order kinetics is the fact that after a sample has been run, the apparent concentration approaches base-line steady state asymptotically, and never actually reaches it. Of course, in practice the base line has been reached whenever it has been approached to within the error with which it can be measured. However, samples can be crowded together so closely by decreasing \(t_{\text{in}}\) that the small but significant apparent concentration by which the signal has failed to reach base-line steady state can add itself to the peak of a subsequent sample. This proves to be the source of interaction between samples (2). The theoretical properties of such interaction can be calculated from the knowledge of \(W_{1/2}\) and \(L\), as follows. First one must make the assumption that the sample concentration is read at the highest portion of the peak just before the pen begins to descend prior to the next sample (which may not always be the case with sequential "phased" instruments). Then, taking the value \(t_{\text{in}}\), subtract from it the value of \(L\), and divide the result by \(W_{1/2}\). The number obtained is the effective number of half-wash times between samples. Obviously, if this number were one, 50% of the concentration of any given sample would appear as part of the following sample. If it were two, 25%; three, 12.5%; etc. By reference to Fig. 8, one can calculate directly what percentage of any given sample will appear as an additive error in the following sample. This figure is the "percent interaction" (2). This calculation has shown reasonable agreement in application to practical cases.

It is also possible to use Fig. 8 to calculate the decrease in precision to be expected theoretically as a result of variability of \(t_{\text{in}}\). If a sampler is improperly made or becomes worn, it may be observed that \(t_{\text{in}}\) will vary from sample to sample, sometimes randomly and sometimes in a systematic fashion. It can also be shown that \(t_{\text{in}}\) may vary as a function of the height of the surface of the sample in its cup. Obviously if \(t_{\text{in}}\) is sufficiently long for 100% of sample steady state to be reached, then variations in \(t_{\text{in}}\) have no effect whatsoever on the concentration read. By reference to Fig. 8, it can be seen, however, that if \(t_{\text{in}}\) were one \(W_{1/2}\), where only 50% of the steady state is reached by the peak height, then a timing error which shortens or lengthens \(t_{\text{in}}\) by 0.1 \(W_{1/2}\) would decrease the percentage steady state reached and, therefore, the apparent concentration to 47% of the steady state or increase it to 53% of the steady state, thus causing a ±6% error in the concentration observed.
One can make similar calculations at any values chosen for $t_{in}$. Figure 9 gives the theoretical relationship between $t_{in}$ and the percentage error. One can therefore calculate, for any measured timing errors, minimal values of $t_{in}$ required to prevent these timing errors from making the precision worse than any chosen value. It should be noted that some sampler models tend to have timing errors which are proportional to time while others have timing errors which are absolute. Figure 9 is calculated for the latter case, but similar calculations are easily made for the former.

It is therefore clear that percentage of sample steady state reached, and timing precision are affected by the value of $t_{in}$ and not by the value of $t_{ba}$, whereas the observed %I can be expected to be affected only by $t_{ba}$ and not by $t_{in}$. This is illustrated by Fig. 10 which relates various values of $W_{1/2}$ to sampling rate (a function of $t_{in}$) and percentage interaction.

The parameter $W_{1/2}$ is a measure of the longitudinal mixing occurring in the conduits of continuous flow analyzers. The monumental contribution of Skeggs to analytic chemistry was to introduce a technic by which $W_{1/2}$ could be lowered to values which made continuous flow analysis
practical—namely the bubble. Attempts to discover the origin of the lag phase have not yet been successful. However, it too is a meaningful parameter for continuous flow analysis. These 2 measurements which can be made on any continuous flow analytic system can be used to express, quantitatively, the characteristics of that system. Thus, improvements in methodology can be expressed as comparisons between $W_{1/2}$ and $L$, before and after the improvements. Similarly, comparisons between instruments performing the same determinations can be made, and monitoring of one instrument over a period of time can be very conveniently done, all in terms of these parameters or their derivatives, e.g., %I. Sudden large variations in these parameters, or deviations beyond certain preset limits can be taken to indicate the necessity for maintenance or repair work on the system. In fact, these parameters seem to provide an extremely sensitive indicator of malfunction for continuous flow analytic systems.

**Conclusion**

With the observation that the longitudinal mixing reactions which occur in continuous flow analytic systems follow first order reaction kinetics with respect to apparent concentration, it becomes possible to
provide easily measured kinetic parameters. These parameters—half-wash time and lag phase—can be used in design and evaluation of continuous flow analytic instruments and methodologies. They provide a practical quantitative theoretical foundation for the rapidly growing field of continuous flow methodology in analytic chemistry.

References