

University of Massachusetts Amherst

From the Selected Works of Julian Tyson

1994

Evaluation of the Precision of the Flow Injection Doublet Peak Method

Julian Tyson
Roger T. Echols



Available at: https://works.bepress.com/julian_tyson/76/

Evaluation of the Precision of the Flow Injection Doublet Peak Method

Roger T. Echols and Julian F. Tyson*

Department of Chemistry, University of Massachusetts, Box 34510, Amherst, MA 01003-4510, USA

The factors affecting the precision of the flow injection doublet peak method are discussed with particular reference to the contribution from the uncertainty associated with the calibration procedure. A single-line flow injection manifold was used in which the concentration gradients were generated by alternating helical reactors and the reaction products were monitored by a simple detector incorporating diodes as light source and intensity transducer. The reactions between lanthanum and Methyl Thymol Blue and between sodium hydroxide and hydrochloric acid in the presence of Bromothymol Blue were used. Optimization studies involved the method of steepest ascent, two figures of merit (the slope of the calibration and the standard deviation of a measured concentration) and two parameters (pump speed setting and the number of segments in the alternating helical reactor). The resulting minimum uncertainties, which ranged from 3 to 9%, were found to be insensitive to changes in experimental parameters. The major contribution to the uncertainty in a determined concentration was found to be the scatter of the points about the regression line fitted to the calibration data. Alternative approaches to flow injection peak width methods are discussed and it is pointed out that the doublet peak method has the unique feature of giving rise to an inherently linear calibration function, because the reference points at which the time interval is measured are not fixed at a particular value of the detector response for all concentrations.

Keywords: Flow injection; doublet peak method; precision; calibration; uncertainty

Introduction

Equations that describe the concentration–time profile of a flow injection (FI) peak have been developed in the past 15 years as a result of work by a number of researchers.^{1,2} Equations that accurately describe the shape of an FI peak under the conditions of laminar flow have not been derived, but the theoretical model and equations that describe the passage of a slug of analyte through a well stirred tank have been developed;^{3–10} these equations form the basis of most of the work in the area of FI peak-width methods, in which a time interval on the concentration–time profile of an FI peak is the analytical parameter of interest.

Early work in this area by Růžička and co-workers^{3,4} established the basis of FI determinations based on time, employing the width of an FI peak instead of peak height as the quantitative parameter of interest. The semi-logarithmic relationship between the time interval between fixed reference points (Δt_{rp}) and concentration was established in this work using a tanks-in-series model reduced to one tank. Determinations were termed ‘continuous flow titrations’,³ a term which has been the source of some debate in the

literature. Various other names for these methods have been proposed: ‘pseudo-titrations’,¹¹ ‘flow injection titrations’¹² and ‘variable-time kinetic methods’.⁵ All methods are subsets of time-based determinations of which most are peak-width methods.

The development of a comprehensive set of equations to describe the concentration–time profile of an analyte in a well stirred tank was completed by Pardue and co-workers in a series of papers.^{5–9} The most recent of these is a thorough review of the previous work.⁹ Equations were derived using a variable-time kinetic model for single- and double-line FI systems, with and without reaction. Experimental situations in which the reactant is in excess were included in the derivation. Calibration equations for time intervals on the leading edge, trailing edge and for the peak width of the FI peak were also derived; in all instances the mathematical relationship between Δt_{rp} and the logarithm of concentration is non-linear. For reference points at equal concentrations on the leading and trailing edges of a peak,

$$\Delta t_{rp} = \left(\frac{V}{Q} \right) \ln \left\{ \left[\frac{(C_s - C_{rp})}{C_{rp}} \right] \left[\exp \left(\frac{V_i}{V} \right) - 1 \right] \right\} \quad (1)$$

Linear relationships are obtained when the reference point concentration is much less than the injected analyte concentration. All symbols used in this paper are defined in Table 1.

Work by Tyson^{10,12} focused on the use of the time interval between doublet peaks as the analytical parameter for time-based determinations. Doublet peaks are obtained when the injected analyte slug is in excess over the reagent stream as the

Table 1 Definitions of symbols

Symbol	Definition
C_s	Concentration of sample
C_r	Concentration of reagent
C_{ep}	Concentration at equivalence point between which time interval is measured
C_{rp}	Concentration at reference point between which time interval is measured
Q	Volumetric flow rate
Δt	Time interval between any two points on concentration–time profile
Δt_{ep}	Time interval between two equivalence point concentrations
Δt_{rp}	Time interval between two reference point concentrations
V	Volume of well stirred tank
V_{eff}	Volume of equivalent well stirred tank for a static mixing device
V_i	Volume of sample injected
b	Slope of calibration plot
n	Number of data points used in the regression
m	Number of replicate measurements of y_0
x_i	Individual x values used to construct calibration plot
y_0	Experimental value from which x_0 is calculated
\bar{x}	Arithmetic mean of x values used to construct calibration plot
\bar{y}	Arithmetic mean of y values used to construct calibration plot
s_{x_0}	Estimated standard deviation of x_0
$s_{y/x}$	Standard deviation of y residuals

* To whom correspondence should be addressed.

slug passes through the detector. Two peaks are observed: one as a result of the reaction between the front boundary of analyte and the reagent stream and the other as a result of the reaction between the rear boundary of the analyte and the reagent. Doublet peaks are not observed if the sample concentration–time profile is monitored; typically, the absorbance of the product of a complexometric reaction or the absorbance of an acid–base indicator is monitored. Equations that describe the passage of the analyte through the well stirred tank were derived for single- and double-line situations. The concept of dispersion was used to develop a linear relationship between the time interval between equivalence points (Δt_{ep}) and the logarithm of concentration:

$$\Delta t_{ep} = \left(\frac{V}{Q}\right) \ln \left\{ \frac{C_s}{C_r} \left[\exp\left(\frac{V_i}{V}\right) - 1 \right] \right\} \quad (2)$$

Equivalence points are a function of concentrations of sample and reagent and are not fixed reference points.

Jordan and Pardue¹³ have recently shown that the agreement between theoretical and experimental concentration gradients in a well stirred tank is excellent. Another paper¹⁴ evaluated different methods of analysing data from an FI experiment. Calibration equations for time intervals taken from different reference points on the FI concentration gradients were evaluated. The effect of changes in experimental variables (such as flow rate) on the use of Δt data was compared with peak-height and peak-area methods.

Recent work by Carroll and Tyson^{15–17} has extended the capabilities of FI doublet peak methods. Inexpensive LED-based detectors have been designed and developed for use in doublet peak methods.^{15,16} A diode laser has also been employed as the light source.¹⁷ A second-generation instrument, the doublet peak detector (DPD), has three LEDs (red, yellow and green) and a diode laser light source. A micro-processor is used to store transmittance *versus* time data for a single injection; the peak maxima are determined and the time interval is output to a liquid-crystal display.

Work by Echols and Tyson¹⁸ focused on the mixing device used to create the concentration gradients in the doublet peak method. A static mixer termed the alternating helical reactor (AHR) was chosen as a suitable alternative to well stirred tanks. The straight-line fit of data (Δt *versus* $\ln C_s$), the relatively large slopes of calibration plots and the ease of construction were reasons for choosing these mixers over knotted reactors, single-bead string reactors and other static mixers. Simple determinations employing the AHR as mixing devices were described and the uncertainty in such determinations was addressed. The standard deviation of a determined concentration,¹⁹

$$s_{x_0} = \frac{s_{y/x}}{b} \left[\frac{1}{m} + \frac{1}{n} + \frac{(y_0 - \bar{y})^2}{b^2 \sum (x_i - \bar{x})^2} \right]^{1/2} \quad (3)$$

was used to calculate confidence intervals that reflected the overall uncertainty of a determination. In the derivation of this equation it is assumed that the errors in y values are uniform and that there are negligible errors in the x values. For this work, all y represent Δt terms and all x represent $\ln C_s$ terms.

Previous work has shown that the confidence limits for doublet peak determinations of zinc and hydroxide ion were approximately $\pm 9\%$ of the known amount of analyte.¹⁸ The percentage differences of determinations (analyte content *versus* analyte found) were within these limits, typically $\pm 5\%$. It was thought that the precision of the method could be improved by an increase in the slope of the Δt – $\ln C_s$ plot. A decrease in $s_{y/x}$ would also improve the precision, but $s_{y/x}$ is a measure of random fluctuations around the regression curve¹⁹ and hence is difficult to optimize. The standard error of the estimate can be decreased by increasing n , the number of data

points used in the regression, but not by setting experimental parameters to optimum values. This work focused on increasing the slope as a means of experimentally controlling the precision.

Experiments describing the optimization of the slope *versus* flow rate and mixing device volume are presented in this paper. The uncertainty of FI peak-width methods is discussed with consideration of these data; some previous misconceptions about the precision of FI doublet peaks are addressed. The two equations, Eqns. (1) and (2), that describe the time interval between doublet peaks (or peak width for a single peak) are evaluated.

Experimental

Apparatus

A single-line flow injection system was used in the optimization experiments. Components of the manifold included a variable-speed peristaltic pump (Ismatec; Cole-Parmer, Niles, IL, USA), a six-port injection valve (Rheodyne; Supelco, Bellefonte, PA, USA), injection loops and various sizes of alternating helical reactors (AHR). The AHRs were constructed by inserting 3/16 in i.d. plastic helical segments into 0.6 cm i.d. tubing. The helical segments are available as 'in-line static mixers' from Cole-Parmer. Slug injection was used in all experiments. Injection loops (1.0 and 1.4 ml) and connecting tubing were constructed from 0.8 mm i.d. PTFE tubing. The DPD has been described elsewhere.^{15,16} A red light-emitting diode was employed as the light source (maximum emission at approximately 625 nm). A square cross-section glass tube inserted into the flowing stream was used as the flow cell; it is estimated that a volume of 1 μ l is interrogated by the light beam.

Reagents

Lanthanum–methyl thymol blue reaction

Lanthanum(III) standards over a range of concentrations from 10.44 ppm (7.520×10^{-5} mol l⁻¹) to 104.4 ppm (7.520×10^{-4} mol l⁻¹) were prepared by dilution of 2626 ppm (1.890×10^{-2} mol l⁻¹) lanthanum chloride stock solution standardized against EDTA. Approximately 3×10^{-5} mol l⁻¹ Methyl Thymol Blue (MTB) solutions were prepared from analytical reagent grade MTB (95% purity). (Aldrich, Milwaukee, WI, USA). All solutions were buffered to pH 6.2 with acetic acid–acetate buffer.

Sodium hydroxide–hydrochloric acid reaction

Hydroxide ion solutions over a range of concentrations from 8.15×10^{-4} to 1.63×10^{-2} mol l⁻¹ were prepared from a 0.345 mol l⁻¹ stock standard solution. An approximately 6×10^{-5} mol l⁻¹ Bromothymol Blue (BTB) solution was used as the reagent stream; the pH of the BTB solution was adjusted to 3.45 with hydrochloric acid.

Procedures

The complexometric reaction between lanthanum and MTB was used in the optimization study. Four replicate injections of each standard were made and the absorbance–time product profile of the reaction was monitored at 610 nm. The volume of the mixing device and the flow rate were the control factors for the slope optimization experiments. In order to avoid experimental complications, the mixer volume was represented by the number of segments in the AHRs and the volumetric flow rate was represented by pump setting on the Ismatec pump. Thus, changes in the variables could be made

in unit steps. The method of steepest ascent was used as the algorithm for the optimization; with the experimental difficulty of not being able to vary either control factor continuously, the simplex method could not be used. The factor space was limited by the volume of the mixer and by the pump setting; it was not practical to use mixers with a large number (>20 , approximately 2.0 ml) of segments because of the time requirements of the detector. Flow rates from pump settings of less than 5 (approximately $11 \mu\text{l s}^{-1}$) were not feasible as a result of pulsing of the flow in the peristaltic tubing.

An initial factor space was established at the edge of the experimental parameters used in previous work¹⁸ (experiments 1–4). Experiments 5 and 6 ‘stepped’ in the directions dictated by the first factor space; a second factor space was established by experiments 7–10. Experiment 11 was performed as a check on the results. The statistics required for eqn. (3), slope, $s_{y/x}$, \bar{y} , Σx and Σx^2 , were obtained using Statview 1.0 (BrainPower, Calabassas, CA, USA). In order to establish confidence intervals for determinations based on doublet peaks, it was necessary to choose an x_0 ($x_0 = \ln C_s$) and y_0 (Δt) to use in eqn. (3). Confidence intervals for the $\ln C_s$ of interest were obtained using the determined s_{x_0} values; the limits of the confidence interval were converted into ppm or molarity, transformed accordingly and expressed as positive and negative percentage differences from C_s . Confidence intervals obtained in this manner will not be symmetric because of the logarithmic function. A value of 15.64 ppm La^{III} ($\ln C_s = 2.750$) was chosen as the ‘unknown’ concentration.

Data from the acid–base experiments were used to confirm results obtained with the La–MTB system. Only five sets of reactions were performed at points in the region of maximum slope. A concentration of $5.248 \times 10^{-3} \text{ mol l}^{-1}$ ($\ln C_s = -5.250$) was used as the ‘unknown’ concentration.

The unknown concentrations were chosen so as to be away from the centroid of the calibration (where the confidence interval is at a minimum) towards both the upper and lower ends of the calibration range.

Results and Discussion

Optimization

Results from the slope optimization are given in Table 2 and displayed in Fig. 1. Approximate volumes and flow rates are listed for reference, but were not used in the optimization. The first factorial (experiments 1–4) dictated stepping in the direction used in experiments 5–7; the second factorial indicated that a plateau had been reached at the edge of the boundaries chosen for the experiment. It is not surprising that the highest slope is obtained for conditions of the largest AHR and the slowest flow rate. It was thought that the effect of one of the two variables on the slope would be greater than

observed, resulting in a different maximum than obtained with experiment 10.

The maximum obtained, a slope of 40.4 s for a 21-segment AHR and a pump setting of 05, is predicted by eqn. (1) for well stirred tank conditions, but not for the flow conditions established in the AHRs. The consequence of the experiment not being performed under well stirred conditions can be seen in an examination of mixers at the same pump setting (e.g., experiments 2 and 4 and experiments 7 and 10). This does not apply universally because there is a marked increase from 15 to 18 segments at a pump setting of 05 (experiments 6 and 7). These results indicate that the condition of slow flow rate is most important in establishing a steep slope. The increase in slope obtained by increasing the length of the mixers by a length of three segments is not as great as the increase in slope with a decrease in flow setting of 1–2 units.

Uncertainty in Determinations

The precision of the FI time-based method is reflected by the percentage confidence limits in Table 1. For the La^{III} standards used in creating the calibration plots for the La–MTB system, the square-root term of eqn. (3) is constant for all experiments (0.599). Hence, in comparing the precision of the experiments, it is the slope and the standard deviation of the estimate that determine whether one set of experimental conditions is better than another. For these data, the s_{x_0} values do not vary much over the response surface. A plot of s_{x_0} versus the number of segments of the AHR and the pump setting illustrates this (Fig. 2).

Application of the F -test (ratio of variances) shows that there is a statistically significant difference between the s_{x_0} of experiment 10 and the s_{x_0} of experiments 9 and 11. For other results no clear trend was visible. It was found that the variation in $s_{y/x}$ as a result of experiments performed over a period of days offsets any increases in the slope. Hence the standard deviation of the determined concentration is relatively insensitive to variations in experimental conditions. For the example concentration listed in Table 2, it is reasonable to expect confidence intervals of 5–9% for determinations based on the time separation between doublet peaks.

The results of the NaOH–HCl experiments were similar to those discussed above (Table 3). The conditions for these experiments were chosen in the region near the steepest slope of the La–MTB experiments. Lower $s_{y/x}$ values resulted in smaller confidence limits. The better fit of the calibration data to a straight line can be attributed to the sharp transitions that occur at the peak maxima in the doublet peaks of acid–base systems and to the greater number of points used in the calibration ($n = 25$). Locating the second doublet peak maximum for a metal–ligand system is more difficult because the second doublet is generally broad. These results are slightly better than those in Table 2; it is reasonable to expect

Table 2 Results from optimization of slope with respect to mixing device volume and flow rate. Lanthanum–Methyl Thymol Blue reaction

Expt. no.	No. of segments	Volume/ ml	Pump setting	Flow rate/ $\mu\text{l s}^{-1}$	Slope/s	$s_{y/x}/s$	s_{x_0}/s	Confidence limits (%) ($C_s = 15.64 \text{ ppm}$)	
1	15	1.6	12	27.7	14.4	0.68	0.0284	–5.90	6.27
2	15	1.6	9	21.0	18.9	1.09	0.0344	–7.12	7.66
3	12	1.3	12	27.6	14.4	0.70	0.0289	–6.01	6.39
4	12	1.3	9	20.8	17.1	1.04	0.0366	–7.55	8.17
5	15	1.6	6	27.6	28.3	1.72	0.0364	–7.52	8.13
6	15	1.6	5	11.6	34.1	1.72	0.0302	–6.27	6.69
7	18	1.9	5	11.5	40.2	1.81	0.0269	–5.61	5.94
8	18	1.9	7	16.1	28.3	1.40	0.0296	–6.16	6.56
9	21	2.2	7	16.0	31.0	1.99	0.0384	–7.91	8.59
10	21	2.2	5	11.5	40.4	1.47	0.0218	–4.57	4.79
11	21	2.2	11	25.3	18.7	1.21	0.0386	–7.95	8.64

an uncertainty in the range of 3–6% for the sample determination of NaOH.

Comparison with Previously Published Work

It is difficult to compare the results discussed above with the work of Pardue and co-workers because of the lack of

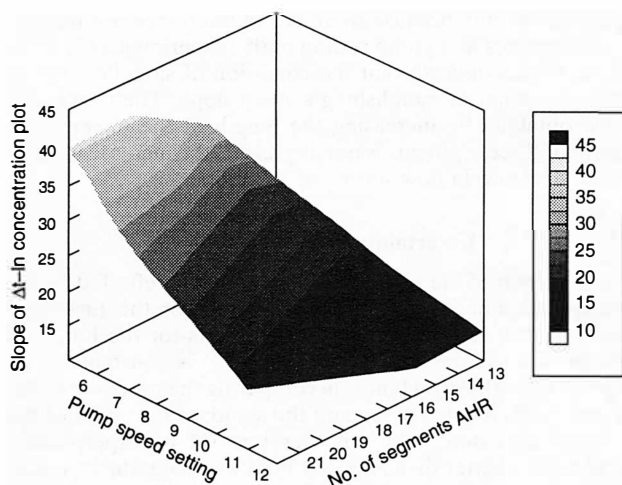


Fig. 1. Three-dimensional plot of the slope of the calibration plot as a function of the number of segments in the alternating helical reactor and the pump setting. The response surface of the slope is described by the shading and numbers in the key.

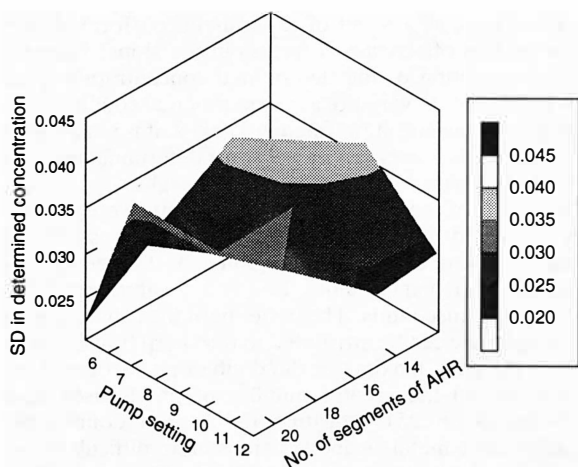


Fig. 2. Three-dimensional plot of the standard deviation (SD) in a determined concentration (s_{x_0}) as a function of the number of segments in the alternating helical reactor and the pump setting. The response surface of s_{x_0} is described by the shading and number in the key. Note the relative insensitivity of the uncertainty as a function of the experimental variables.

Table 3 Results from NaOH–HCl/BTB experiments

No. of segments	Pump setting	Slope/ s	$s_{y/x}/s$	s_{x_0}/s (on $\ln C_s$)	Confidence limits (%) ($C_s = 5.248 \times 10^{-3}$ mol l^{-1})	
18	7	22.5	0.58	0.0141	–2.87	2.96
18	5	32.2	1.07	0.0180	–3.66	3.80
15	7	22.3	1.05	0.0256	–5.15	5.43
15	5	31.0	0.96	0.0169	–3.44	3.56
21	6	29.2	1.06	0.0198	–4.01	4.18

regression data in the literature. Pardue and Fields⁶ discussed the determination of HCl with KOH. For a 1.644 ml well stirred tank operating at $41.3 \mu\text{l s}^{-1}$, a slope of 41.7 s and an $s_{y/x}$ of 0.40 s were reported for a plot of Δt versus $-\ln C_a$ [Fig. 3(A) in ref. 6]. The value of s_{x_0} for these data would be an improvement of a factor of two over the values reported in Table 2. Pardue and Jager⁷ reported better straight-line fits for the reaction of triiodide and thiosulfate with electrochemical detection. A slope of 47.4 s and $s_{y/x}$ of 0.29 s for a plot of Δt versus $\ln C_s$ are a factor of 6–8 better than the doublet peak results reported above. The confidence limit for such a system would be less than 1%, an improvement on the results in Table 1 and 2.

Although the comparison of uncertainties of the two methods indicates that the single-peak method is more precise than the doublet peak method, some of the conclusions reached in a previous paper⁹ are misleading. There the authors correctly derived an equation for relative concentration error (*i.e.*, a parameter relating to the accuracy of the method), but then substituted into this values for parameters relating to precision. However, their conclusion, namely that the over-all uncertainty in the doublet peak method can be very high, is not disputed. If the treatment developed here is applied to the data published in ref. 10, the confidence intervals about calculated concentration values would be close to 100% owing to the small slope and the scatter of the limited number of points about the regression line.

Previous work has shown that some static mixing devices (such as large-diameter tubes or coiled tubing) are not suitable alternatives to the well stirred tank because of the relatively flat slope of the Δt – $\ln C_s$ plots. The concept of the ‘apparent’ or ‘effective’ well stirred mixing chamber volume has been used to describe this.¹⁸ The effective volume is the volume required to provide the same slope at the same flow rate ($V_{\text{eff}} = bQ$). AHRs behave qualitatively as well stirred tanks, but have V_{eff} that are 2–3 times less than the actual volumes of the mixers. Some of the static mixers¹⁸ had V_{eff} that did not increase with an increase in real volume. That was the case for the mixer used in the preliminary work on doublet peaks;¹⁰ hence it is inappropriate to compare those data with data obtained with a well stirred tank. Alternating helical reactors were chosen as a suitable alternative mixing device to the well stirred tank because the effective volume (and slope) could be increased to values that would reduce the large uncertainty observed in the early work.

Comparison of Δt Equations

Eqns. (1) and (2) have been the source of some confusion over the past decade on the issue of linearity of the Δt – $\ln C_s$ plot.^{9,10} The dispute has been academic, because the equations are the same; either equation can be derived from the other equation using the relationship

$$C_{\text{ep}} = C_{\text{rp}} = \frac{C_s C_r}{(C_s + C_r)} \quad (4)$$

where C_{ep} and C_{rp} are equivalence points and reference points on the FI peak.^{9,10} Both equations have been used as the theoretical basis for obtaining a linear Δt – $\ln C_s$ relationship.^{6,7,10} For eqn. (2) the linear relationship is exact, whereas for eqn. (1) it is appropriate for situations in which the reference point concentration is much less than the sample concentration ($C_{\text{rp}} \ll C_s$). The latter assumption does not affect the uncertainty in time-based FI methods as evidenced by the results discussed above.

The validity of eqn. (2) has finally settled on the question of the validity of the assumption that the product profile mimics the sample (reactant) profile. This assumption is important because equations used in obtaining eqn. (2) are derived from

an injected sample with no reaction and as a result of the experimental requirement that the product profile must be monitored. Slow chemical reactions¹³ and diffusion effects⁹ have been cited as the reasons why the assumption is not valid. The rate of the reaction is not a factor for the chemical systems studied to date. Simulations have shown that the rate constant for a reaction does not have to be very large (k of at least 1.0 s^{-1} for a first-order reaction) in order for the product profile to match the theoretical sample profile (for no reaction). There has been no evidence that the effects of diffusion invalidate the linear Δt - $\ln C_s$ relationship that is obtained with the doublet peak work. Further, the effect of molecular diffusion on the over-all dispersion in the mixing chamber is negligible in mixing devices in which the flow is significantly disrupted. This is the case with well stirred tanks and with the AHRs.

In using the single-peak method, a fixed reference point concentration is chosen to establish the detector response between which the time interval is measured.^{6,7} Pardue and Jordan⁹ described several alternative approaches for measuring Δt_{rp} on different parts of the concentration-time profile. In the doublet peak method, the doublet peak maxima are the reference point concentrations and are also the equivalence point concentrations. In the typical experiment a range of C_s are injected into a constant C_r ; C_{ep} increases slightly with increasing C_s [see eqn. (4)]. The important thing to note is that C_{rp} are not fixed; they are established by the chemistry of the system, rather than being measured at a fixed concentration. This is a feature unique to the doublet peak method and is the reason why the mathematical relationship between Δt_{ep} and $\ln C_s$ is linear without approximation.

Overview of Doublet Peak Method

The focus of our recent work has been on practical rather than theoretical aspects of time-based FI methods of analysis.^{9,15-18} For reasons of simplicity and for the fundamental reason that doublet peaks will not form in double-line manifolds, a single-line manifold was used in these experiments. Work in establishing solid-state detectors and using static mixing devices has simplified the experimental requirements of the method.¹⁵⁻¹⁷ Solid-state detectors cannot be used in the same manner if Δt is measured at reference points at the base of FI peaks because of the drift of the LED and diode laser light sources. Detection is further simplified by the ease with which the variable reference points (doublet peak maxima) can be located with an integrator or simple microprocessor. The large linear dynamic ranges reported earlier are still a feature of doublet peak methods. However, as the number of orders of magnitude in a Δt - $\ln C_s$ plot increases, there is a substantial decrease in precision. The results presented above indicate that it is not necessary to operate under experimental conditions that result in the steepest slope; hence it is reasonable to expect to be able to obtain two orders of magnitude in a calibration plot without a great decrease in precision.

Conclusions

The 95% confidence limits of FI doublet peak determinations incorporating alternating helical reactions and an LED-based

detector are in the range 3–9% around the true value. This is as good a precision as can be expected under existing operating conditions and is much better than that previously attributed to the doublet peak method. Experimental conditions of large mixer volume and slow flow rate result in a maximum slope, but this does not decrease the uncertainty in a given determination because of the scatter around the fit of data to the Δt - $\ln C_s$ relationship ($s_{y/x}$). The response surface for a plot of uncertainty versus flow rate and AHR length is reasonably flat. Operating conditions that yield a lower slope but do not sacrifice precision are advantageous in increasing the number of orders of magnitude covered by one calibration.

Previous conclusions about eqns. (1) and (2) are correct. The important differences in the equations arise as a result of the reference points: to use eqn. (1), time intervals are measured at a fixed reference point; to use eqn. (2), time intervals are measured between the time between doublet peaks, which are variable reference points. The use of the latter method of determining Δt_{ep} results in a linear relationship between the time interval and the logarithm of the concentration of injected sample.

Financial support from Pfizer (Groton, CT, USA) is gratefully acknowledged.

References

- 1 Růžicka, J., and Hansen, E. H., *Flow Injection Analysis*, Wiley, New York, 2nd edn., 1988.
- 2 Valcarcel, M., and Luque de Castro, M. D., *Flow Injection Analysis: Principles and Applications*, Ellis Horwood, Chichester, 1987.
- 3 Růžicka, J., Hansen, E. H., and Mosbaek, H., *Anal. Chim. Acta*, 1977, **92**, 235.
- 4 Ramsing, A., Růžicka, J., and Hansen, E. H., *Anal. Chim. Acta*, 1981, **129**, 1.
- 5 Pardue, H. L., and Fields, B., *Anal. Chim. Acta*, 1981, **124**, 39.
- 6 Pardue, H. L., and Fields, B., *Anal. Chim. Acta*, 1981, **124**, 65.
- 7 Pardue, H. L., and Jager, P., *Anal. Chim. Acta*, 1986, **179**, 169.
- 8 Jager, P., and Pardue, H. L., *Anal. Chim. Acta*, 1986, **187**, 343.
- 9 Pardue, H. L., and Jordan, J. M., *Anal. Chim. Acta*, 1989, **220**, 23.
- 10 Tyson, J. F., *Anal. Chim. Acta*, 1986, **179**, 131.
- 11 Stewart, K. K., *Anal. Chim.*, 1983, **55**, 931A.
- 12 Tyson, J. F., *Analyst*, 1987, **112**, 523.
- 13 Jordan, J. M., and Pardue, H. L., *Anal. Chim. Acta*, 1992, **270**, 195.
- 14 Jordan, J. M., Hole, S. H., and Pardue, H. L., *Anal. Chim. Acta*, 1993, **272**, 115.
- 15 Carroll, M. K., and Tyson, J. F., *J. Chem. Educ.*, 1993, **70**, A210.
- 16 Carroll, M. L., and Tyson, J. F., *Anal. Chim. Acta*, in the press.
- 17 Carroll, M. K., and Tyson, J. F., *Appl. Spectrosc.*, 1994, **48**, 276.
- 18 Echols, R. T., and Tyson, J. F., *Anal. Chim. Acta*, in the press.
- 19 Miller, J. C., and Miller, J. N., *Statistics for Analytical Chemistry*, Ellis Horwood, Chichester, 2nd edn., 1988.