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# HIGH ACCURACY PRE-FORMED GRADIENT DISCONTINUOUS FLOW TITRIMETRY

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**Summary**—A variant of Discontinuous Flow Analysis (DFA) titrimetry is described in which a pre-formed gradient is established between two titrant concentrations with the analyte aspirated at a constant rate into the gradient The gradient encompasses a narrow range of analyte concentration (10% variation in this instance), and provides a high encoder pulse resolution A simple acid–base titration model using photometric endpoint detection achieved comparable accuracy to conventional batch titrimetry (approximately 0 1% relative), with excellent calibration linearity ($r^2 = 0\ 9997$, standard error of estimation approximately 0 05% relative over six standards) Titrations were performed at the rate of one every 25 sec, with 0 8 ml of analyte and 0 85 ml of titrants consumed The method is fully automatic

Fluid-based analytic methods have been classified as either batch or flow. Batch methods are known for high precision and amenability to verification processes such as standard additions; whereas flow methods have the advantages of small sample volumes, fast analysis and ease of automation. Modern batch analysis generally employs automated piston burettes to transfer volumes of samples, reagents, diluents, *etc.* to a measurement cell. The volumes dispensed are a function of piston displacement, and the measurement is static. In the newly developed flow method of Discontinuous Flow Analysis (DFA)[1–5] measurements are a function of combinations of piston velocities and accelerations. Predetermined and highly reproducible flow rate ratios of analyte to reagent with effective mixing mean this dynamic approach offers the prospect of combining the advantages of batch and flow methods in a single method. Previous publications have shown that DFA can use volumes at least as low as 800 $\mu$l;[1] can operate at high analysis speeds (6 seconds per cycle)[1], can operate fully automatically[6] and can perform incremental standard additions analogous to batch methods.[7] However, for a piston pump flow method such as DFA to satisfy the requirements of a unified method, it has to be capable of matching the precision and accuracy of batch methods. In this study, a titrimetric application involving a linear gradient was chosen, to demonstrate this particular aspect of DFA.

The ability to discriminate between small differences in concentration is a prerequisite for high precision and accuracy. Automatic batch titrators are pre-eminent in this regard. These use stepper motor drive piston burettes to deliver titrant into a known quantity of sample. Usually, the delivery rate is controlled by feedback from the sensor, for instance, a pH electrode in the case of acid–base titrations. Resolution is not critically dependent on sensor performance, and is primarily a function of high stepper pulse counts.

In the titrimetric applications of DFA reported to date[1–5] cam-actuated piston pumps

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provided a continuously varying flow rate ratio of titrant $F_T$ to sample $F_A$, such that:

$$F_T + F_A = F_S, \quad (1)$$

where $F_S$ is the suction pump flow rate, which is held constant (Fig. 1A).

For a simple monoprotic acid–base titration, the endpoint occurs when:

$$C_A \cdot F_A = C_T \cdot F_T, \quad (2)$$

where $C_A$ and $C_T$ are the concentrations of analyte and titrant, respectively,

$$C_A = C_T \cdot F_T/(F_S - F_T) \quad (3)$$

Since $C_T$ and $F_S$ are constants, the resultant inverse calibration curve (Fig. 1A) favours resolution at low $C_A$.

This assumes instantaneous mixing and detection at the confluence of titrant and analyte streams. In practice, the detected endpoint position ($D_E$) is delayed by a constant which relates to the ratio between the volume from the confluence point to the detector, to the total volume which passes the detector during the titration half cycle.

In a typical example, standard deviations varied between 0 2 and 1.4%; and accuracies between <0 01 and 10% [4] The titration range (that is, the lowest and highest measurable analyte concentration with respect to a particular titrant concentration) was either 4:1 to 1·4[1] (a factor of 16), or 2:1 to 1:2[3] (a factor of 4), depending on the predetermined geometry of the particular cam profile. Clearly, the larger the range, the lower the resolution in analyte concentration and hence the lower the precision and accuracy. It would be expected that reducing the range would improve the resolution. However, in practice, this translates to a shallower cam profile, and the system becomes increasingly sensitive to small irregularities in the profile.

An alternative approach is to establish (that is, pre-form) a gradient between two titrant concentrations (say from 100% to 0% for Titrant 1, and 0% to 100% for Titrant 2) and aspirate the analyte at constant rate (Fig. 1B). A steep gradient cam profile can then be used, which minimizes the effect of small profile irregularities. The range can be easily varied by the choice of the titrant concentrations. Maximum resolution is obtained by reducing the difference in concentrations of the titrants to the minimum necessary for a selected group of samples.

With reference to Fig. 1B:

$$F_{T1} + F_{T2} = K_1 \quad \text{(a constant)}. \quad (4)$$

At the endpoint, $C_A \cdot F_A = C_{T1} \cdot F_{T1} + C_{T2}\ F_{T2}$

$$C_A = (C_{T1}\ F_{T1} + C_{T2}(K_1 - F_{T1}))/F_A \quad (5)$$

Since $F_A$, $C_{T1}$, and $C_{T2}$ are all constants for a given group of titrations,

$$C_A = K_2 \cdot F_{T1} + K_3$$

($K_2$ and $K_3$ are constants). (6)

That is, the relationship between $C_A$ and $D_E$ will be linear for a linear gradient between $T_1$ and $T_2$. Addition of another fluid stream at a constant rate (for example, an indicator, as in this case) will offset the $D_E$s by another constant.

The system is calibrated using a minimum of two analyte standards. Since the same titrants are used for both calibration and sample analysis, it is unnecessary to know their concentrations with any degree of accuracy. Moreover,

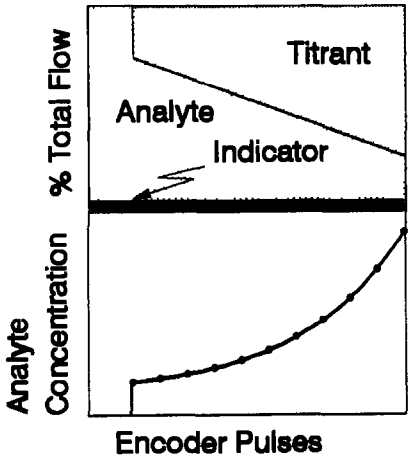


Fig 1A Relationship of titrant to analyte in conventional DFA titration

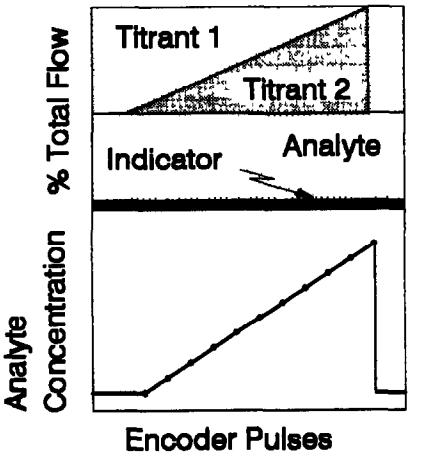


Fig 1B Relationship of titrant to analyte in pre-formed gradient DFA

some effects which can cause a difference between the equivalence point and the endpoint of conventional titrations may be eliminated. For example, high concentrations of indicators are not recommended for conventional titrations where say, a strong acid is titrated against a standardized base because the acidic or basic character of the indicator itself will cause an error. In the present system, as in a previously reported DFA titration,[3] any indicator effect on the endpoint will shift the $D_E$s of standards and unknowns equally, that is, the differences between equivalence points and endpoints are constant for all titrations. This permits the use of relatively high concentrations of indicator, and consequently a very short optical path. This has the following advantages: (a) the change in intensity of transmitted light at the endpoint is large, so that a simple, inexpensive and relatively insensitive detector may be used, (b) compared to a long optical path, the significance of sample absorbance is reduced,[3] and (c) the very small cell volume is less prone to bubble entrapment

## EXPERIMENTAL

### *Batch titrations*

A Metrohm 686 Titriprocessor (Herisau, Switzerland) with a 665 Dosimat and 20 ml burette was employed. Titrations were performed in a thermostatted vessel at $25 \pm 0.1$°C containing a pH electrode (model IJ 42, Ionode Pty. Ltd., Brisbane, Australia), and the contents purged with nitrogen. The "Monotonic Equivalence Titration" mode was found to be the most satisfactory for this work, and conditions for optimal endpoint detection were determined by preliminary titrations. For example, a time delay of 15 sec was set between titrant additions around the endpoint, based on observations of the time response of the system. The sodium hydroxide titrant was prepared by diluting a concentrate ("Convol", BDH Chemicals, Poole, U.K.); the container was protected by a guard tube filled with fresh soda lime. Potassium hydrogen phthalate served as standard, and "unknown" concentrations were prepared from known masses made to volume with $<15$ $\mu$S conductivity water.

### *Pre-formed gradient DFA titrations*

A schematic of the DFA apparatus is shown in Fig. 2. The pistons of the three pumps on the right side of the cam are all fixed to a moveable frame, which is driven at a constant velocity by the larger segment of the cam. After passing the detector, the waste fluid stream comprising reacted titrants, analyte and indicator, is drawn into suction pump P7 at a constant flow rate. The smaller segment of the cam drives the left frame according to the flow profile in Fig. 1B. From 24° to 157° of cam rotation, the pistons of pumps P2 and P3 undergo a uniform acceleration, but in opposite directions. The concentration gradient between Titrant 1 and Titrant 2 is formed by discharging Titrant 1 from pump P5 and simultaneously removing some of it from the fluid stream at a uniformly increasing rate by means of the Titrant 1 suction pump P3. As Titrant 2 pump P2 is actuated by the same cam segment as P3, it is discharging at exactly the same rate at which Titrant 1 is being withdrawn. Thus the total titrant flow rate is constant, and equal to that of P5. The sample is aspirated at a constant flow rate equal to the difference in flow rates between P7 and (P5 + P6). All pistons are made of precision ground and polished ZTP zirconia (Sapphire Engineering Inc, Maryland, U.S.A., and Maret SA, Bole, Switzerland) with spring-loaded piston seals of graphite-impregnated Teflon (Bal Seal Engineering Inc, California, U.S A.). The piston diameters are: P2 and P3 4.763 mm, P5 6.35 mm, P6 3.175 mm, and P7 9.525 mm. The piston stroke length is 25 00 mm. The calculated volume of aspirated sample is 792 $\mu$l, with 445 $\mu$l of each titrant consumed. The encoder generates 5000 pulses per cam revolution, and the cam speed was set at a conservative 25 sec cycle, which comprises one titration and one refill/discharge operation. Solution temperatures were $25 \pm 0.5$°C during the determinations. The three-way solenoid valves used in conjunction with the pumps (model LFYA2418032H, Lee Inc., U.S A.) generate heat when energized. However, they are only energized during the refill/discharge half cycle, and there was no evidence of significant heat transfer in the titration half cycle, which could affect the results. All connecting tubing is 1 mm ID Teflon.

A cross-section of the combined mixer/detector is shown in Fig. 3. The four inlets, meet at right angles immediately under the mixing chamber (Fig. 3, Section A–A). The 10 mm diameter mixing chamber contains a 9.3 mm diameter "star head" PTFE spin bar (Nalgene, U.S.A.), which is coupled to an external magnet

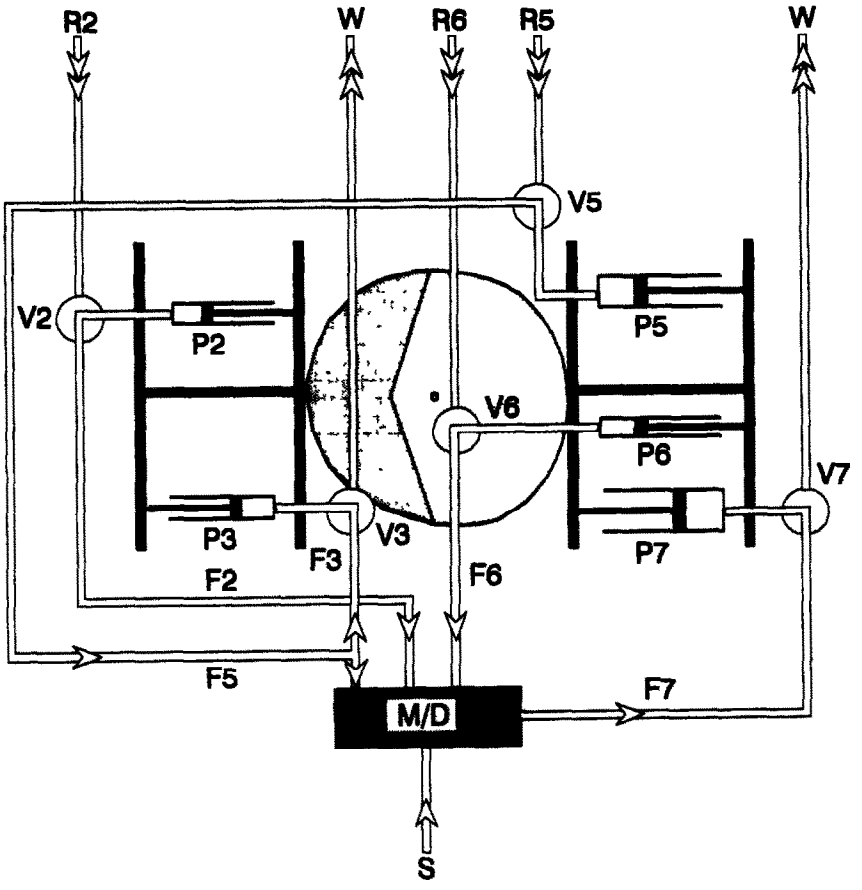


Fig 2 Schematic of DFA in pre-formed gradient titration mode Pn = pump n, Vn = valve n, Fn = fluid line n, S = sample, W = waste, M/D = mixer/detector, single arrow = flow direction, first half-cycle, double arrows = flow direction, second half-cycle, R2 = Titrant 2, R5 = Titrant 1, R6 = Indicator

rotating at approximately 500 rpm. The free volume of the mixing chamber is approximately 350 $\mu$l. The mixed solution enters a short section of glass tube (8 mm OD, 1.2 mm ID), which has a yellow LED (590 nm, 5 nm bandpass) and an opposing phototransistor mounted transverse

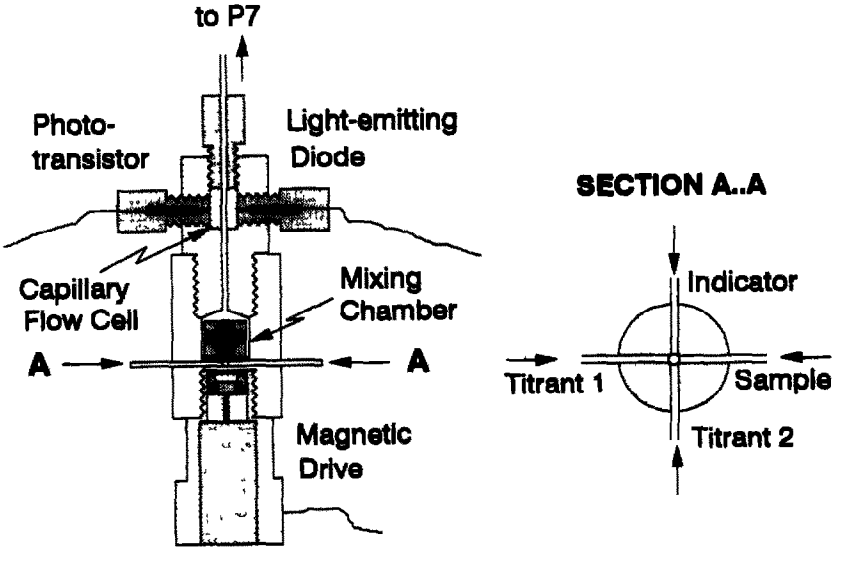


Fig. 3 Mixer/detector cell (cross section)

Table 1 Determination by batch and pre-formed gradient DFA titrations

| Batch titration | | | | |
|---|---|---|---|---|
| Analyte KH Phthalate | Prepared ($M$) | Determined ($M$) | Relative error (%) | Relative standard deviation (%) ($n = 6$) |
| "Unknown 1" | 0 1000 | 0.10009 | 0 09 | 0 03 |
| "Unknown 2" | 0.0900 | 0 09014 | 0 15 | 0.02 |
| **Gradient DFA titration** | | | | |
| Analyte HCl | Prepared ($M$) | Determined ($M$) | Relative error (%) | Relative standard deviation (%) ($n = 6$) |
| Standard | 0 1010 | — | — | 0 02 |
| Standard | 0 1020 | — | — | 0 04 |
| Standard | 0 1030 | — | — | 0 01 |
| Standard | 0 1050 | — | — | 0 05 |
| Standard | 0.1070 | — | — | 0 03 |
| Standard | 0.1080 | — | — | 0 01 |
| Standard | 0.1090 | — | — | 0 04 |
| "Unknown 1" | 0 1043 | 0.1042 | 0 10 | 0 07 |
| "Unknown 2" | 0 1062 | 0 1061 | 0 09 | 0 03 |
| Mean of standard deviations | | | | 0 03 |

to the tube axis. The transmitted light intensity is recorded against encoder pulse.

A dedicated "Windows"-based software program was written to control operational parameters such as cycle speed(s), and valve positions; and to receive data. This was collected in ASCII files (2500 data points per titration half cycle) and later processed with a spreadsheet program (Quattro Pro 4.0, Borland International Inc., California, U.S.A.).

An arbitrary analyte range of 10% variation was selected for this study. Sodium hydroxide titrants of approximately 0.1000 and 0.1100$M$ were prepared from the "Convol" concentrate. Hydrochloric acid standards of 0.1010, 0.1020, 0.1030, 0.1050, 0.1070, 0.1080, and 0.1090, and "unknowns" of 0.1043 and 0.1062$M$ were prepared by dilution of 1.000$M$ acid ("Univol", Ajax Chemicals, Sydney, Australia), using the piston burette. A 0.5% solution of the sodium salt of bromothymol blue was used as indicator (Ajax Chemicals, Sydney, Australia).

## RESULTS AND DISCUSSION

### *Batch titrations*

This experiment proceeded in two parts: the standardization of the sodium hydroxide against known masses of potassium hydrogen phthalate, (mean of six titrations 0.09991 $\pm$ 0.00002$M$); then the use of this titrant to measure the concentration of the "unknowns". Effectively, the accuracy of the titration system was tested. The results are presented in Table 1.

### *Pre-formed gradient DFA titrations*

Figure 4A illustrates the raw data (curve P) around the endpoint for a typical titration, with a curve (Q) generated from the weighted moving average (period = 100) of this data. The second derivative of $Q$ was used to calculate the relative standard deviations of $D_E$s from the mean (Table 1). In Fig. 4B, curve R was obtained by averaging each data point from six individual titrations, subjecting this data to a weighted moving average treatment (period = 100), and taking every 10th data point; from which the second derivative curve (S) was obtained. This method was found to significantly improve the $r^2$ of the calibration, compared to an approach where endpoints of individual curves (such as curve Q) were averaged.

The mixing cell is responsible for some reduction of "flow noise" which causes small irregular variations in the component concentrations in the fluid stream, and to which the indicator/detector system is acutely sensitive. Preliminary experiments with a reed mixer[1] showed a near vertical response at the endpoint. However, this was accompanied by visible colour banding; evident as sharp peaks at the endpoint. Although the reed mixer performed well in some less critical DFA applications,[1,4] its inability to average flow irregularities in the direction of flow precluded its use in this work. The present mixer substantially overcame this problem. This was partly a function of the averaging effect caused by the comparatively

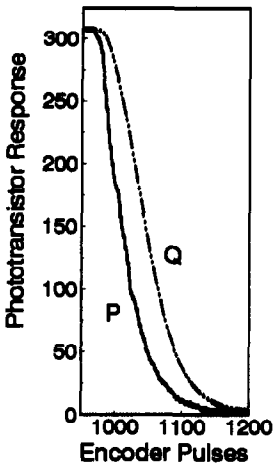


Fig 4A Effect of weighted moving averaging on raw titration data (0 1010$M$ HCl)

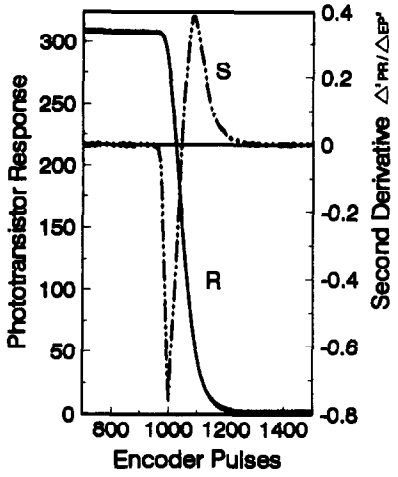


Fig 4B Titration curve (0 1010$M$ HCl) and calculated second derivative

larger swept volume of the cell. It was also found to be highly effective at inducing turbulent mixing in the fluid stream travelling in the narrow space between the mixer and the cell wall. The titration curves suffer some attenuation of slope as a consequence, but are sufficiently steep for a second derivative treatment to be satisfactory for determining the end point. Figure 5 is the calibration of $D_E$s of the analyte standards and the "unknowns". It will be noted that the set of $D_E$s has undergone a positive encoder position displacement with respect to the gradient profile (Fig. 1B), principally because of the offset described earlier As previously stated, the concentrations of the two titrants need not be known exactly. However, significant mismatching with the desired range of analyte concentrations will result in less than optimal use of the encoder pulse "window" of the gradient. In the present system, the window is $2500 \times (157\text{–}24)/180 = 1847$ encoder pulses. The position and extent of the measured analyte range with respect to the window depends on the actual concentrations of titrants compared to the standards. A compression effect is evident in Fig. 5 in that the extrapolated experimental range extends from $D_E = 926$ pulses at 0.100$M$ to $D_E = 2185$ pulses at 0.110$M$, a difference of 1259 pulses (cf. 1847). Due to various offset effects, the highest standard (0.109$M$) is positioned around the extreme of the gradient window, where a rapid change to a single titrant takes place (Fig. 1B). This region is ill-defined because of diffusion effects in the measuring cell at this point. Consequently, the $D_E$ is displaced from the curve The net result is that in the present case, the practical range of analyte concentrations is reduced to a variation of 8%. Excluding the 0.109$M$ standard, the linear correlation coefficient ($r^2$) of the curve was 0.9997, and the standard error of estimation 0.00005$M$. Since the "unknowns" were prepared in exactly the same manner as the standards, it is not necessary to know their absolute values, and for the purpose of this study, the accuracy of the method alone can be assessed This is similar to that of the batch determination, that is, approximately 0.1% (Table 1).

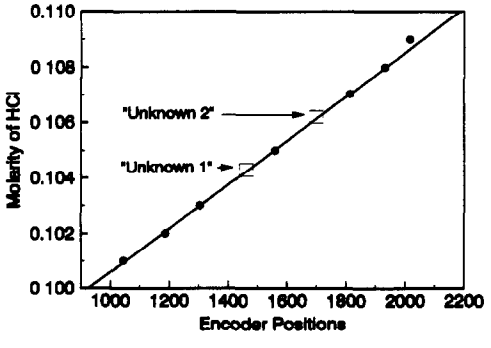


Fig 5 Calibration curve for pre-formed gradient DFA titrations

Table 1 also shows precisions of the analyte standards and the "unknowns" The best precision is similar to the batch method; the worst significantly higher. However, the latter does not exceed the absolute error for both methods (approximately 0.0001$M$). The variation in precision appears random, and observation of the mixing cell during the titrations generally revealed that the lower precisions were related to the entrapment of small air bubbles on the

underside of the spin bar. Future work will include improvements in the mixer design. Six titrations were completed in $2\frac{1}{2}$ min, which is about the actual titration time of the batch titrations, but much less than the total time which included manual pipetting of the sample. An improvement in precision consistency is required if the speed advantage inherent in DFA is to be realized, that is, a single titration suffices for the determination. It is considered that precisions of $\pm 4$ encoder pulses ($\pm 0.00003M$, applied to the present work) should be routinely possible, and future studies will be directed to achieving at least this objective. Clearly, the present encoder pulse resolution is adequate for such work; further improvement will depend on an understanding of the "flow noise", and the role of the mixer.

Batch titrimetry can operate over a large range, but resolution is fixed and directly related to the burette volume. The range of pre-formed gradient DFA can be matched to the expected variation in sample concentration, and may be considerably reduced from the arbitrary 10% chosen for these experiments. Batch titrators do not need calibration, the titrant normally requiring at most a single standardization. Pre-formed gradient DFA requires an initial calibration based on at least two standards, preferably more. However, regular recalibration should be unnecessary if the titrant concentrations are unchanged, because the piston drive is invariant and the detector response is reliable. This has been demonstrated in an industrial application of conventional DFA titrimetry,[6] where regular checks over a three month period showed no change in the calibration curve.

Batch titrators may be fully automated, with such features as automatic sample dilution and dispensing, reagent addition, and washing of the titration sensors and vessels; however, such systems are invariably very costly, and complex in the number of sub systems involved. They are often relatively slow, and may offer little over semi-automated systems on an analysis per unit time basis.

Pre-formed gradients which are fast, linear, and highly precise can be used for other purposes, such as calibration.[8] Preliminary work in this area has commenced.[9] Gradients can also be used for speciation, for example, by pH, solvent composition, temperature, redox potential, *etc.* As well, polyfunctional gradients involving two or more parameters can easily be formed by the above method. As the present apparatus has provision for eight piston pumps, two independent linear gradients may be formed with three extra pumps to operate sequentially or concurrently. The ability to hold one or more parameters constant (say, indicator concentration, as in the present case) while simultaneously varying others will enable the study of complex chemical reactions with particular relevance to biochemical analysis.

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