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Solid-state microprocessor-controlled detector for doublet peak measurements in flow-injection analysis

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Abstract

A solid-state detector for time-based transmission measurements in flow-injection analysis (FIA) based on a light-emitting diode (LED) source and photodiode (PD) transducer with microprocessor controller is described. The instrument components cost less than US$ 500. A simple peak-finding algorithm was capable of accurate measurements of the time interval between doublet peaks (Δt). Comparison of results, obtained using the detector, with theory for an acid–base reaction with bromothymol blue indicator is made. Linear relationships between the measured Δt and the logarithm of the injected concentration were obtained for two systems whose injection volumes were 987 and 1650 μl. The correlation coefficients, based on 14 and 13 data points, were 0.99_9 and 0.99_2, respectively. The detector could measure transmittance signals corresponding to 0.00098 absorbance above the baseline.

Key words: Flow injection; Solid state microprocessor-controlled detector

1. Introduction

Considerable effort has been made in the development of inexpensive flow-injection systems for research and pedagogical use. Hansen and Ruzicka [1] developed a modular system for use in teaching laboratories shortly after flow-injection analysis (FIA) was introduced. Davis [2] has presented an extremely inexpensive detector, constructed in-house, and of relatively crude design, for use in a variety of laboratory situations. Recently, Dasgupta et al. [3] reviewed the literature on LED-based detectors for absorption measurements. In that article, they described the advantages and disadvantages of various instrumental components (LEDs vs. laser diodes, photodiodes vs. phototransistors) and configurations, and stressed the need for a reference-based instrumental design, in order to avoid long-term instrumental drift [3].

The use of time-based measurements obviates both the need to eliminate or compensate for long-term drift of the source or detector, and the requirement that the signal have a linear response to concentration [4]. Ordinarily, FIA measurements are based on peak height. Doublet peaks, which form if the analyte is in excess at the profile center when the sample bolus reaches the detector, are seen as disadvantageous, limiting

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the upper range of the determination. However, by choosing appropriate manifold lengths and reagent concentrations such that doublets occur for even relatively low concentrations of analyte, the analytical chemist can expand the working range of the determination [4].

If the assumption is made that dispersion occurs only in the mixing chamber of the manifold, in accordance with that of a single well-stirred tank, the time between the two peaks, Δt, can be related to a number of system parameters by the following equation:

$$\Delta t = (V/Q) \ln\{[S][\exp(V_i/V) - 1]/[R]\} \quad (1)$$

where V is the volume of the mixing chamber, Q is the flow rate, [S] is the concentration of analyte in the sample injected into the manifold, [R] is the concentration of reagent in the carrier stream, and V_i is the volume of sample injected [4]. Hence, a plot of Δt versus ln[S] will be linear. Moreover, the relative error introduced in determination of unknown concentrations arising from the precision of the Δt measurements and the logarithmic relationship can be small in comparison with the uncertainty introduced from the calibration process [5].

Dasgupta and Loree [6] have developed instrumentation for peak width measurements in FIA and liquid chromatography (LC). Previous work by our group involved the development of a simple instrument for doublet-peak measurements suitable for use in teaching laboratories [7]. This instrument had as its optical sources LEDs and as detectors PDs; the output from the PD was plotted on an integrator, and Δt values were calculated based on the difference in recorded time of each peak [7]. In an effort to streamline the process, and to develop an instrument that is both more versatile and more easily portable than the first iteration, a microprocessor has been incorporated into the new instrument. The present instrument, referred to here as the doublet peak detector (DPD), has the FI manifold, detector electronics, microprocessor controller and readout contained within the same unit. It can be used as a self-contained instrument, or interfaced with a computer or an oscilloscope.

2. Experimental

2.1. Flow-injection manifold design

A schematic of the DPD is shown in Fig. 1. It is desirable to protect the electronic components from being flooded if a leak develops within the manifold system; hence, manifold components and the detector module are located below the electronic components in a project box ($13 \times 25 \times 30$ cm, McKinstry Metal Works). The majority of the manifold components are located within the DPD unit as illustrated in Fig. 1A.

A Rheodyne 6-port valve is mounted in the front side of the project box; small segments of flow tubing (0.9 mm i.d., Chemplast, Chemfluor® Teflon® spaghetti tubing) are used to connect the ports for injection loop, sample introduction, and waste output to unions mounted in the container wall. For purposes of testing the performance of the DPD, a simple, single-line manifold was chosen, of the type used in our previous studies [7,8]; however, the DPD has additional ports for use in more complicated manifold configurations. The mixing component used is a 60-cm length of flow tubing, and is more tightly coiled than that used in the previous LED-based instrument, in order to better approximate a well-stirred tank [7]. This component is connected to the detector module by a short segment of flow tubing.

Alteration of the manifold components housed within the DPD is facile, but it is not practical to do so within an experiment because components are anchored with cable ties. Because sample loop volume is used commonly as a variable in flow-injection experiments, the sample loop is connected externally to the box through two unions, which lead to valve ports. This has one disadvantage: it allows a certain amount of room light to enter the DPD through the tubing, resulting in a higher background signal. Significant problems with light leakage were not noted with this system; however, it would be possible to reduce stray light ingress by use of opaque tubing.

Likewise, because flow rate is commonly controlled by changing pump tubing or pump settings during the course of an experiment, the peri-

staltic pump is not contained within the DPD. Pump tubing is connected through a union to one of the valve ports. In the work presented here, we used a variable-speed peristaltic pump (Ismatec); however, a less expensive pump could be substituted.

Samples are injected into the sample loop using a disposable plastic syringe, which connects to a union on the side of the DPD unit. Waste from the injection valve and from the detector effluent is transported out of the DPD through additional unions.

2.2. *Calibration of manifold components*

The volume of each sampling loop was measured by filling the loop with 0.0464 M NaOH, injecting this sample into a carrier stream of deionized distilled water, collecting the effluent, and titrating with 0.0130 M HCl, employing a suitable acid–base indicator. Three replicate measurements were made for each sample loop. Flow rates for the solid-state instrument manifold were calculated from the time necessary to fill a 10.00-ml calibrated flask. Three replicate measurements were performed for each flow rate.

2.3. *Transmission detector design*

Three ultrabright light-emitting diodes (LEDs, Newark Electronics) are used as detector source elements, one of each of the following colors: red (630 nm), yellow (585 nm), green (565 nm), where each LED has a bandwidth of approximately 30 nm and the wavelength indicated is the wavelength of maximum emission. PIN photodiodes (PDs, Newark Electronics) were used as the detection components. The LEDs and PDs were mounted perpendicular to the direction of flow in appropriately sized holes in a small block of aluminum, as illustrated in Fig. 1A. The LEDs are not glued into place, but fit snugly into the drilled holes. The active area of each PD is centered across the flow cell from the corresponding LED,

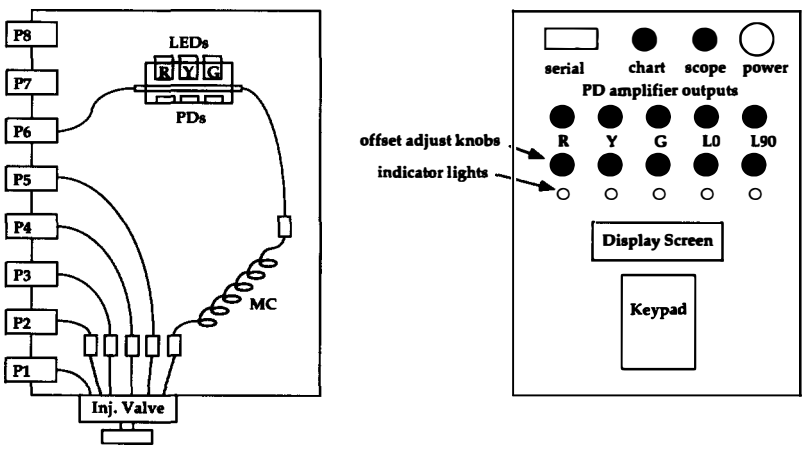

Fig. 1. (A) Inside view of manifold components in doublet peak detector (DPD) unit. Inj. valve is a six-port rotary valve; MC is the mixing component, a coiled segment of flow tubing. PDs are the photodiode detector elements; each is paired with a light-emitting diode (LED) source. R, Y, G refer to the positions of the red, yellow and green LEDs. P1-P8 are union ports into the DPD. In the present study, a single-line manifold is used and the following connections have been made: the sample loop is connected between P1 and P2; injection syringe attaches to P3; the flow from the peristaltic pump enters through P4; P5 and P6 carry waste from the valve and detector, respectively, out of the DPD. P7 and P8 are not used, but could be employed in more complicated manifold designs. (B) Top view of DPD unit. The labelled circles are BNC connectors. R, Y and G refer to the outputs from photodiodes paired with red, yellow, and green LEDs, respectively. L0 and L90 are outputs from PDs to be paired with a laser diode for transmission and fluorescence measurements, respectively.

resulting in improved optical alignment over the previous detector version [7]. Because the case of each PD is connected to positive (not ground), and it is not desirable to have the cathodes of the three PDs in electrical contact with the aluminum block and each other, the PD cases are wrapped in Teflon tape prior to insertion into the detector module. The corners of the detector module are attached to the base of the DPD unit with silicone sealant.

The flow cell is a piece of square-cross-section capillary tubing ($1 \times 1 \times 50$ mm, Wale Apparatus); it is connected to the flow tubing by a short segment of pump tubing (red/red), and the connection is sealed with clear silicone as described previously [8]. Square-cross-section tubing was used in order to have a fixed path length in the cell, and to allow the detection of smaller signals. The initial design used flow tubing as the flow cell [7]; such tubing absorbed and scattered significant amounts of light in the visible region (as evidenced by its blue-gray color) and, because it was circular in cross section, the path length varied across the cell. In the current design, path length is 1 mm and is uniform across the cell; the sampled volume of the flow cell at each LED/PD detector pair is approximately 1 μl.

2.4. *Controller design*

The heart of the microprocessor unit is a single-chip microcomputer (DS5000, Dallas Semiconductor). It is programmed in code to collect and store the data, calculate the time between the two peaks in the doublet (in the range 10–240 s), and display the results to the nearest 0.01 s. The sampling frequency is 1 kHz.

The circuitry used to power the LEDs, perform current-to-voltage conversion of the signal from the PDs, and to offset the output voltage is modified slightly from that described previously [7]. It differs from the earlier circuit design in the voltage reference for the offset circuitry; complete circuit diagrams may be obtained from the authors. The object of the offset is to bring the background signal to zero volts, so that the gain settings may be used to the fullest extent. The instrument user sets the offset, while pumping

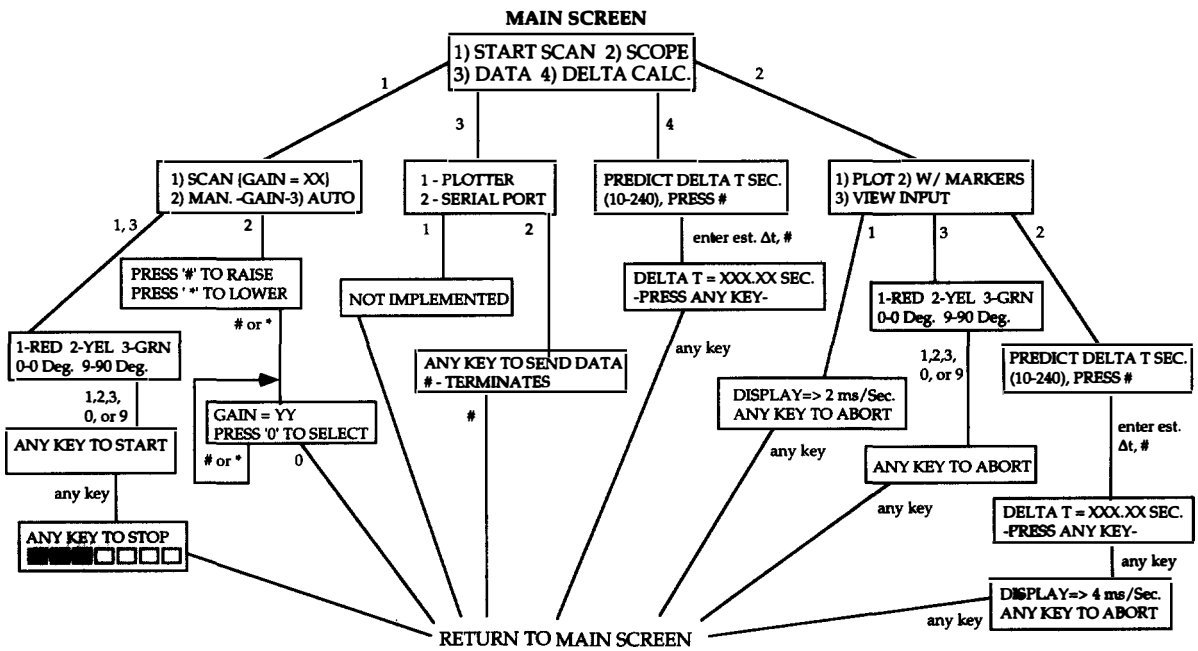

Fig. 2. Flow chart of operation of doublet peak detector (DPD). Note that when the data is displayed, a certain number of milliseconds of readout on the oscilloscope corresponds to 1 s of doublet peak data (designated ms/Sec. in the flow chart).

carrier stream through the DPD; a separate control for each detector PD is located on the instrument top panel (Fig. 1B).

Each PD has a fixed gain of 10^6 V/A in the current-to-voltage conversion step. Additional signal amplification (by a factor of 2^n, $n = 0$–7) is performed in the microprocessor.

After the signal has passed through the current-to-voltage converter, it is sent to an 8-bit analog-to-digital converter (AD7569, Analog Devices). The data is digitized and stored in memory for the duration of the experiment. The offset, amplified signal can be output to a stripchart recorder, integrator, or other readout device through a BNC connector if the user desires to view the amplified (but unprocessed) signal.

Detailed circuit diagrams may be obtained from the authors. The total cost of the electronic and manifold components used to construct the DPD is less than US$ 500.

2.5. *Controller operation*

Fig. 2 is a flow chart of the operation of the microprocessor controlled DPD unit. The keypad is used to enter numbers for the various selections. There are four selections on the main screen: (1) collect a new data set, (2) view most recently collected data on oscilloscope (this can include calculation of Δt), (3) send stored data to plotter or serial port of a computer, (4) calculate Δt. A data file is kept in memory until the next set of data is collected.

Data collection with the DPD unit consists of several steps. First, the user selects a gain setting or chooses autogain. Next, the source/detector combination to be monitored is chosen: RED, YEL and GRN refer to red, yellow, or green LED/PD combination, respectively; 0 Deg and 90 Deg refer to PDs placed 180° and 90° from a diode laser source (not described here) for transmittance-based or fluorescence-based measurements, respectively. The user then injects the sample into the flowing carrier stream and presses a key to start data collection. The signal is displayed as a bar graph (see Fig. 2); as the sample passes through the detector, a peak in the signal (dip in transmittance) is indicated by a rise in the number of illuminated blocks in the bar graph. After the second peak is observed, the user can stop the data collection; otherwise data collection continues until 240 s have elapsed since the start of the run. If the autogain setting was chosen, the microprocessor collects the data, then calculates the gain setting that should be employed to maximize the observed signal; however, it does not amplify the data. Consequently, if the signal is small, the data collection should be repeated using the gain setting selected by the microprocessor and given in the gain window as "1) SCAN {GAIN = XX}", where XX is the gain factor (2^n, $n = 0$–7).

Once it has collected the data, the microcomputer uses a simple algorithm for the double peak detection (more complicated algorithms could be programmed into the DPD). The user inputs an estimate of the time between peaks, Δt_{input}, within the range of 10–240 s. Each data point is compared to the previous one and the first peak is located; then, the microprocessor waits $\Delta t_{input}/2$ s before beginning to compare data points again. It locates the second maximum in the same way as the first, and then computes the time between the two peaks and displays the calculated Δt on the screen.

The output of the DPD can be sent to a plotter, to an oscilloscope, or in ASCII binary to a computer. If the data is sent to the oscilloscope, the user has the option of displaying tick marks that indicate the points chosen by the controller as the two peaks of the doublet.

2.6. *Reagents used*

The solvent used for all solutions was distilled, deionized water. For the purposes of characterizing the performance of the DPD, the same simple acid–base reaction with indicator that was used in previous work is employed [7]. The carrier stream contained 1.0×10^{-5} M HCl and 6.0×10^{-6} M bromothymol blue (BTB) indicator, and had the characteristic yellow color of the acid form of BTB. Sample solutions of NaOH were prepared by dilution of a 0.5 M stock solution with appropriate amounts of water. Alkaline solutions of BTB are a deep blue in color, and

absorb significantly at the wavelengths emitted by the LEDs used here; the acidic form of BTB does not absorb appreciably at these wavelengths.

2.7. Experimental procedure to test performance of DPD

The carrier stream was allowed to flow through the DPD. The red LED has the best overlap with the absorbance maximum of the blue form of BTB; thus, the signal from the red-LED/PD pair was monitored. The offset for the red LED/PD pair was adjusted manually to zero volts. A sample of NaOH solution was injected into the carrier stream, resulting in an on-line titration. Because the NaOH is in excess in the profile center when the sample bolus reaches the detector, the transmittance of the stream was observed to dip before and after the center of the sample bolus (doublet "peaks" if the signal is inverted, as was done in this circuitry, or if transformed to units of absorbance). The Δt calculated by and displayed on the DPD was recorded manually. To verify whether the identification of the two peaks, and therefore the calculation of Δt, was successful, the output of the DPD was displayed with markers on an oscilloscope (Hameg, Model HM205-3). Any discrepancy between the Δt recorded on the oscilloscope and that calculated by the DPD was noted. Three to five replicate measurements were made for each sample solution; the flow rate was fixed at 1.32 ml/min.

Five sample solutions, with concentrations of NaOH ranging from 1.10×10^{-5} M to 1.10×10^{-1} M, were used. Two different sample loops were tested, one 987 μl in volume, the other 1.65 ml in volume.

3. Results and discussion

With the exception of the data for the 1.10×10^{-5} M NaOH, the Δt values recorded by the DPD were all within 3 s of the Δt measured from the oscilloscope screen ("true" Δt); the first peak was identified correctly, but noise spikes near the second peak were often chosen by the microprocessor as the second peak, resulting in erroneous Δt values. Accurate location of the first peak is simplified by the fact that the first peak is relatively sharp for this particular chemical system; however, the second peak is broad. Doublet peak shapes vary for different chemical systems; a thorough study of doublet peak shapes for a variety of chemical systems has been performed [9].

Peaks observed for the least concentrated solution of NaOH studied were misidentified by the DPD because of the relatively large noise spikes observed, and because no smoothing of the data took place before peak identification. The signal-to-noise ratio was sufficiently large that precise measurements of Δt could be made when the DPD output was displayed on an oscilloscope screen. Modification of the microprocessor code so that the algorithm contains some level of smoothing would eliminate this shortcoming of the DPD.

The DPD is sensitive to small changes in absorbance; for the 1.10×10^{-5} M NaOH sample, the peak signal (least transmission) observed for the BTB indicator was equivalent to 0.00098 absorbance. The major source of electronic noise in the detection circuitry appears to be from the microprocessor circuitry. Improved shielding of the microprocessor from the detection electronics should result in improved signal-to-noise ratio, and allow the DPD to be used for samples that absorb even less strongly than those studied here.

Experimental results were in good agreement with Eq. 1. For the 987 μl sample loop, the plot of Δt (in s) versus [NaOH] (in M) gave a good fit to the line: $\Delta t = 107.4(\pm 0.6) + 7.8(\pm 0.2) \cdot \log[\text{NaOH}]$, $r^2 = 0.99_9$. (The values in parentheses are standard deviations of the intercept and slope, respectively; 14 points were used in the correlation.) Measured values of Δt ranged from 18.8 to 92.4 s. The working range covers four orders of magnitude of NaOH concentration. It should be noted that this fit includes the data for the 1.10×10^{-5} M solution of NaOH. The DPD calculations of Δt for solutions of this concentration were erroneous, so the Δt measured from the oscilloscope tracing was used; if these data points are omitted, there is no significant change in the curve-fit line. For solutions of the other

concentrations, Δt values calculated by the DPD were used, even though more accurate results could be obtained from the oscilloscope. Three replicate injections of a 6.60×10^{-4} M solution were made. The average calculated value of Δt was 51.4 s, with standard deviation 0.9 s; this corresponds to a sample concentration at the 95% confidence interval of 8.28×10^{-4} M > [NaOH] > 6.13×10^{-4} M (data treatment as in [5]).

Dispersion does occur in the sample loop, as well as in the mixing component, which is simply a more tightly coiled segment of the same tubing used in the rest of the manifold; hence, we would expect deviations from theory to be more pronounced as the size of the sample loop increases. Use of the larger sample loop, 1.65 ml, resulted in data that fit reasonably well to the line expected by theory, but the fit is not as good as that for the smaller sample. For this set of 13 data points, $\Delta t = 150(\pm 2) + 1.6(\pm 0.6) \cdot \log[\text{NaOH}]$, $r^2 = 0.99_2$. Values of Δt ranged from 42.5 to 127.9 s. As can be seen from these data, changing the volume of sample injected has a relatively small effect on the slope of the line, but a large effect on the Δt for a particular sample concentration.

The doublet peak detector (DPD) has several advantages: it is a relatively sensitive detector, inexpensive to construct, portable and rugged, and is suitable for many chemical systems whose products absorb light in the green to red region of the spectrum. The main limitations of the current version of the DPD are the short path length, the wavelength limitations imposed by the use of LEDs as light sources, and the current algorithm used to identify peaks and calculate the time between the two peaks in the doublet.

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