

Full paper

A Novel Type of Tri-Colour Light-Emitting-Diode-Based Spectrometric Detector for Low-Budget Flow-Injection Analysis

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Abstract: In this paper we describe a low-cost spectrometric detector that can be easily assembled in a laboratory for less than €80 with a minimal number of optical components and which has proved sensitive and flexible enough for real-life applications. The starting point for the idea to construct this small, compact low-cost spectrometric detector was the decision to use a tri-colour light-emitting diode (LED) of the red-green-blue (RGB) type as a light source with the objective of achieving some flexibility in the selection of the wavelength (430 nm, 565 nm, 625 nm) but avoiding the use of optical fibres. Due to the dislocation of the emitters of the different coloured light, the tri-colour LED-based detector required an optical geometry that differs from those that are described in literature. The proposed novel geometry, with a coil-type glass flow-through cell with up to four ascending turns, proved useful and fit for the purpose. The simplicity of the device means it requires a minimal number of optical components, i.e., only a tri-colour LED and a photoresistor. In order to make a flow-injection analysis (FIA) with the spectrometric detector even more accessible for those with a limited budget, we additionally describe a low-cost simplified syringe-pump-based FIA set-up (€625), the assembling of which requires no more than basic technical facilities. We used such a set-up to test the performance of the proposed spectrometric detector for flow-injection analyses. The tests proved its suitability for real-life applications. The design procedures are also described.

Keywords: Spectrometric Detector, Tri-Colour Light-Emitting Diode (LED), Flow-Injection Analysis (FIA), Syringe Pump, Low-Budget Instrumentation.

1. Introduction

Even though flow-injection analysis (FIA) with spectrometric detection cannot be considered an expensive analytical technique, the expenses are not negligible for someone who has to invest in the basic equipment. Because of the popularity and widespread use of this analytical technique, and also because a FIA with spectrometric detection involves several important fundamental chemical and physical concepts, first- and second-year university students and even pre-university students can benefit a great deal from the opportunity to work experimentally in this field. Unfortunately, however, budget restrictions may be an obstacle for schools, preventing them from being able to offer their students hands-on experience with this important analytical technique.

The objective of this paper is to describe a low-cost spectrometric detector that can be easily assembled in the laboratory with the minimum number of optical components, and which is sensitive and flexible enough to get students acquainted with the fundamental concepts of FIA and also be suitable for real-life applications. In order to make the FIA with a spectrometric detector even more accessible to institutions or individuals on a low budget, we also propose a low-cost simplified FIA set-up, the assembling of which requires no more than basic technical facilities. We used such a set-up when testing the performance of the proposed spectrometric detector for a FIA.

Spectrometric detectors for an FIA based on LEDs emerged soon after LEDs became commercially available. The high degree of simplification of the instrument, the small size, the negligible warm-up time, the low power consumption and the absence of significant warming of the sample are the major advantages of these systems. In 1973 an LED-based photometer with a 30-cm glass flow-through cell and a phototransistor as the sensor was proposed by Flaschka *et al.* [1]. With this photometer the light passed through the cell in the longitudinal direction. In 1978 a detector with a U-type flow-through cell – similar to that of Flaschka *et al.*, but with a path length of 30 mm – was adopted and tested, not only for spectrometric, but also for refractometric measurements in an unsegmented continuous-flow analysis [2].

Cells with different geometries were proposed and investigated in the years that followed. Among these were the following: a cross-configuration cell [3]; a double-beam version of the radial-beam-path flow cell with a reference channel through which the untreated sample passes before the addition of reagents and passage through the sample cell [4]; a Z-geometry flow cell [5], used in a chromatographic system; a liquid drop-cell detection arrangement for FIA [6]; and an optical-fibre-coupled LED-based absorbance detector with a reflective flow cell [7].

The need for multi-wavelength LED-based devices has led to different solutions. In 1990 Trojanowicz *et al.* used a commercially available photometer with a multi-diode light source and sequential switching of the diodes with different emission maxima for a simultaneous flow-injection determination of the aluminium and zinc in alloys [8]. A dual-wavelength detector based on a bi-colour LED was described two years later by Huang *et al.* [9]. Liu *et al.* reported on the coupling of the light from two separate LEDs into a single cell with bifurcated optical fibres [10]. A multi-LED photometer that employs a fibre-optic coupler to guide the light from up to seven LEDs into a single measuring cell was proposed by Hauser *et al.* [11]. The cell consists of a black Perspex body into which the fibre is inserted; it has a 1-cm path length and a cell volume of 8 µl.

The starting point for the construction of the small, compact low-cost spectrometric detector which we propose was the decision to use a tri-colour light-emitting diode (LED) of the red-green-blue (RGB) type as the light source, with the objective to achieve some flexibility in the selection of the wavelength (430 nm, 565 nm, 625 nm), but avoiding the use of optical fibres. The main characteristic of a 5-mm RGB-type LED is that it comprises four light emitters, which are all arranged in a plane in the form of a cross with edge distances of a few millimetres. The two emitters of blue light are positioned opposite each other, and the emitters of the red and the green light are also opposite each other. Due to the dislocation of the emitters of the different-coloured light the tri-colour LED-based spectrometric detector required an optical geometry that differs from those that are described in the literature. In this paper we propose and test the novel optical geometry of an empirical spectrometric detector in which the flow-through cell in the form of a miniature glass capillary coil with up to four ascending turns is positioned between the tri-colour LED and the photo-resistor so that the light of any selected light emitter – blue, green or red – passes vertically through the coil in its axial direction. No additional optical components were used, which contributes to the simplicity, robustness and relatively small size of the spectrometric detector. The basic characteristics of the spectrometric detector and a simplified low-cost FIA set-up, which we additionally propose and used for testing the detector's performance, were defined and evaluated, and their suitability for real-life applications was tested. The prototyping procedures are also described.

2. Results and Discussion

2.1. Optical geometry of the tri-colour LED-based spectrometric detector

In order to select the appropriate optical geometry for the spectrometric detector the optical beams emerging from a tri-colour LED were examined a distance of 3 mm away from the LED's epoxy body. Spots with a circular shape were observed for the green and red light. Both had an area of highest light intensity with a diameter of approximately 8 mm; however, even at this relatively small distance the centres of the two circles were 3 mm apart. The beam of blue light had an elliptical shape. The ellipse with the highest blue-light intensity was 11 mm long and was perpendicular to the line in which the spots of the red and the green light lay. It was clear that all three beams overlap in a circular region with a diameter of 5–6 mm. In order to use the light from all three light emitters effectively and to obtain an appropriate light path length, but to avoid an excessively large internal volume of the flow-through cell, we decided to test a novel glass coil-type flow-through cell and the optical geometry of the spectrometric detector, which is presented in Figure 1. The detector can be easily constructed in a laboratory. The procedure for designing the coil-type flow-through cell and the assembling of the electronic circuitry are described in the Experimental section, 3.1. The design containing an unreferenced LED source and the signal linearly related to the transmittance was used. The emitters of the light of three different colours are switched on separately. The possibility to regulate their luminous intensity using three 10-k Ω potentiometers (Figure 13) proved essential for achieving the optimal sensitivity of the spectrometric detector under different experimental conditions. Three prototyped coil-type flow-through cells were selected for further tests. Their main characteristics were defined and are summarized in Table 1.

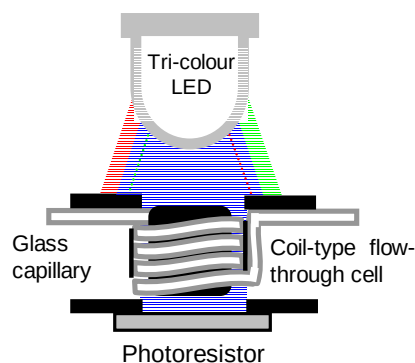


Figure 1. Optical geometry of the tri-colour LED-based spectrometer.

Table 1. The main characteristics of the selected glass coil-type flow-through cells.

	10a cell	11a cell	12 cell
Coil's outer diameter	4 mm	5 mm	6.5 mm
Coil's internal volume	20 μL	27 μL	41 μL

Linearity tests were performed with all three coil-type flow-through cells (10a, 11a and 12) and for all three spectral ranges of the spectrometric detector. The electronic circuitry of the spectrometric detector (Experimental section 3.1) was designed so that it allowed for independent regulation of the red, green or blue light's intensity, thus making it possible to evaluate the light intensity's influence on the detector's response. Linearity tests were performed at the maximum light intensities and the intensities that for each particular case were just high enough to allow for the setting of 100% transmittance at the highest amplification setting of the operational amplifier when the flow-through cell was filled with a blank. Due to the fact that the light of a tri-colour LED of the RGB type with emission maxima at 625, 565 and 430 nm, respectively, and related spectral line half-widths of 45, 30 and 65 nm is not strictly monochromatic, substances with absorption spectra that effectively overlap with the emission spectra of the light emitters had to be selected for the detector's performance test.

A 3-mmol/L potassium manganate(VII) solution proved to be a convenient stock solution for experiments in the range of the green light. Working solutions were prepared by further dilutions with deionised water, which was also used as a blank in the spectrometric measurements. The results of the linearity tests in the range of the green light are presented in Figure 2. The correlation coefficients demonstrate that the proposed spectrometric detector with a new coil-type flow-through cell exhibits good linearity in the range extending at least as far as the absorbance value 0.4. It should be mentioned that in this empirical detector with a specific optical geometry multi-wavelength absorption occurs in the wavelength range where the absorption spectrum of the absorbing species overlaps with the emission spectrum of the selected light emitter; therefore, it is not possible to compare these values with those obtained with a single-wavelength absorption in monochromator-based detectors. The results also prove that the light intensity has a significant effect on the detector's response, and a lower light

intensity allows for a higher sensitivity. The highest sensitivity was observed for the cells with the lowest and highest internal volumes, i.e., 10a and 12. Cell 11a performed moderately well at the lowest light intensity, with the most significant improvement in the response being recorded when lowering the light intensity, if we compare it with the other two cells.

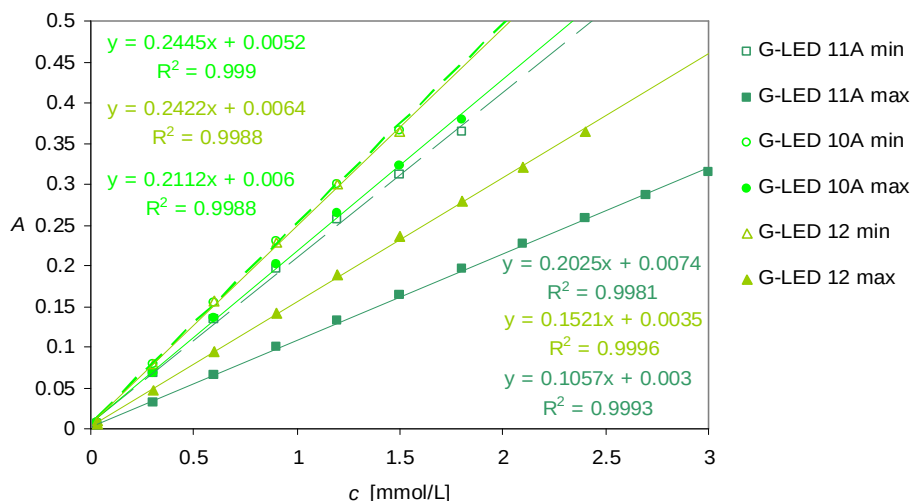


Figure 2. Linearity of the detector's responses in the range of the green light (G-LED) for cells 10a, 11a and 12 at minimum (min) and maximum (max) light intensity.

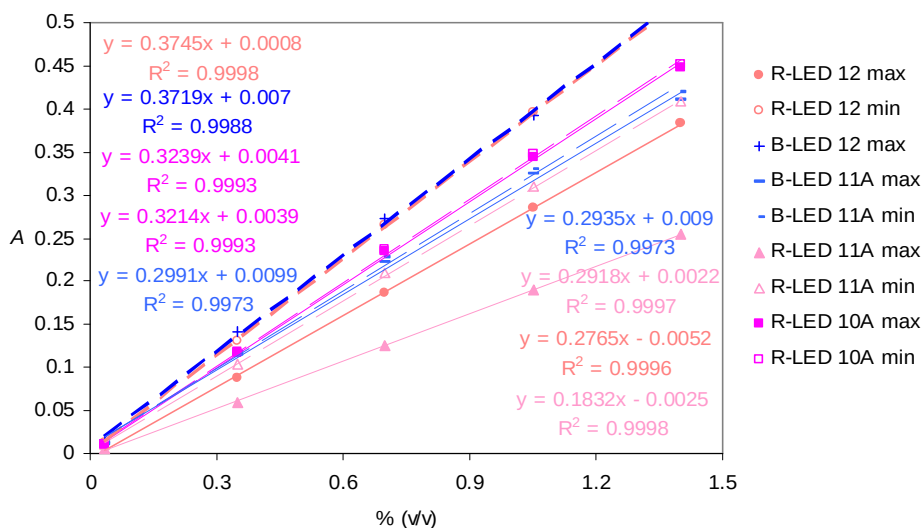


Figure 3. Linearity of the detector's responses in the range of the blue light (B-LED) and the red light (R-LED) for cells 10a, 11a and 12 at minimum (min) and maximum (max) light intensity.

A stock Solution I (Experimental section 3.3) proved to be a suitable combination of food dyes, E102, E110 and E131, for the detector's performance test in the range of the blue and red light because for this solution a spectrum with two wide, well-separated absorption peaks of similar height with absorption maxima at 418 and 662 nm, respectively, is obtained. For the test of the linearity the stock solution was further diluted with 20 mmol/L of ammonium acetate. The measurements were performed

with all three coil-type flow-through cells against a 20-mmol/L ammonium acetate solution as a blank. The results are presented in Figure 3. The absorbencies in the range of the blue and red light are linearly related to the concentration up to the absorbance value of 0.45. This result is very similar to that obtained with the green light. The highest sensitivity was achieved with flow-through cell 12, and it was very similar for both the blue and red light. However, it should be pointed out that with this cell the spectrometric detector was at the limit of its ability in the range of the blue light and only the maximum light intensity with the highest amplification setting of the operational amplifier produced 100% transmittance. This is not at all surprising if we take into account the tri-colour LED's characteristics. The maximum luminous intensities reported by the producer are 90 mcd for red, 70 mcd for green and 12.5 mcd for each of the two blue light emitters. Consequently, an experiment in the blue-light range was not possible at all with cell 10a. This coil's diameter is obviously too small, preventing it from catching enough light to achieve 100% transmittance for the blank. Even in the range of the red light there was only just enough light due to the fact that the lines obtained for the 10a cell at the maximum and minimum light intensities nearly overlap. Here, again, the detector with flow-through cell 11a performed moderately well. Very similar responses were obtained in the blue-light range and at the minimum intensity of the red light.

The 11a coil-type flow-through cell with the external coil diameter of 5 mm and the internal volume of 27 μ L proved to be the best compromise for spectrometric measurements in all three wavelength ranges, allowing for the most satisfactory optical geometry of the spectrometric detector. The highest sensitivity of the spectrometric measurements is obtained at light intensities that are not much more than just high enough for a 100% transmittance setting at the highest amplification setting of the operational amplifier. Linear responses can be expected for absorbance values below 0.45 if the absorption spectrum of the light-absorbing *species* overlaps sufficiently with the emission spectrum of the selected light emitter.

Next, the repeatability of the spectrometric measurements using the proposed detector with the 11a coil-type flow-through cell was tested. Two solutions prepared from the stock Solution I – a 0.7% (v/v) solution and a 1.05% (v/v) solution – were used for the repeatability test in the range of the blue and red light respectively. For the repeatability test in the range of the green light a 2-mmol/L solution prepared from stock Solution II was selected. Relative standard deviations of 0.42%, 0.64% and 0.32% were obtained at mean absorbance values of 0.2181 ($n=12$), 0.2321 ($n=12$) and 0.2290 ($n=11$) for measurements in the range of the blue, red and green light, respectively. The detector exhibits good absorbance-measurement repeatability.

2.2 Performance of the low-cost FIA with the tri-colour LED-based spectrometric detector

Determination of the dispersion coefficients

In order to make a FIA with the spectrometric detector even more suitable for low-budget circumstances, we additionally propose a low-cost simplified FIA set-up with time-based injection (Figure 4), the assembling of which is described in full detail in the Experimental section 3.2. We used such a set-up when testing the performance of the proposed spectrometric detector for flow-injection measurements. In order to test the operation and flexibility of the FIA set-up with the spectrometric

detector the dispersion coefficients D , as introduced by Ruzicka and Hansen [12], were determined for different injection volumes and for different experimental conditions by comparing the absorbance maximum of an injected dye with the absorbance obtained when the spectrometric cell is completely filled with the dye solution. D is a measure of the dilution of the injected samples and is defined as the ratio C^0/C^{max} , where C^0 is the analyte concentration in the original solution and the C^{max} corresponds to the maximum of the peak on injection.

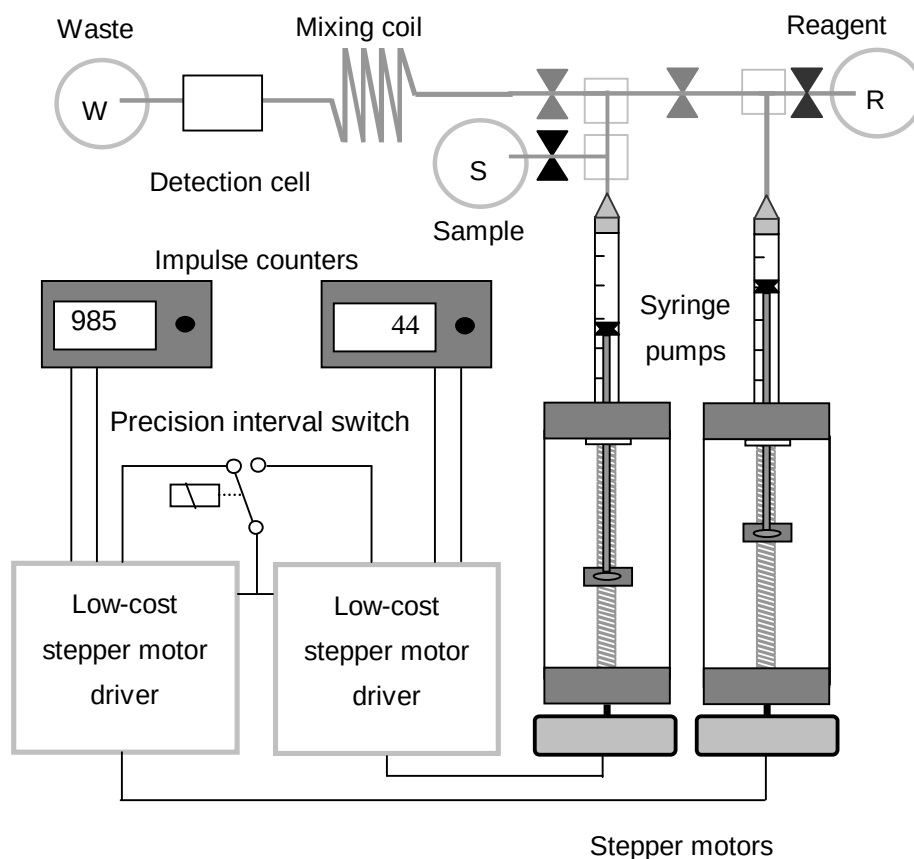


Figure 4. The low-cost simplified FIA set-up with time-based injection, the assembling of which we suggest for low-budget circumstances and which we used for testing the proposed spectrometric detector for real-life applications in the blue and red light ranges.

For the determination of the dispersion coefficient a solution containing E 131 dye was selected, since its absorption spectrum, with an absorption maximum at 638 nm, effectively overlaps with the emission spectrum of the red-light emitter of the spectrometric detector. The working dye solution was prepared by combining 3.5 ml of the dye stock Solution I and 6.5 ml of the 20-mmol/L ammonium acetate solution. Using a flow rate of 56 $\mu\text{L}/\text{min}$ and time-based injection the dye solution was injected into the main line, which contained the 20-mmol/L ammonium acetate solution. The injection volumes ranged from 8.3 to 41.3 μL . The main-stream pump automatically started propelling the liquid when the injection period had elapsed. Experiments were performed at two different main-stream flow rates, 1.5 mL/min and 0.5 mL/min, with and without the mixing coil (coil tube length of 35.2 cm, with 16 turns). The 10a spectrometric cell (20 μL) was the one that was mainly used, but one of the experiments was

repeated with cell 12 (41 μL) in order to estimate the effect of the cell's internal volume on the dispersion coefficients.

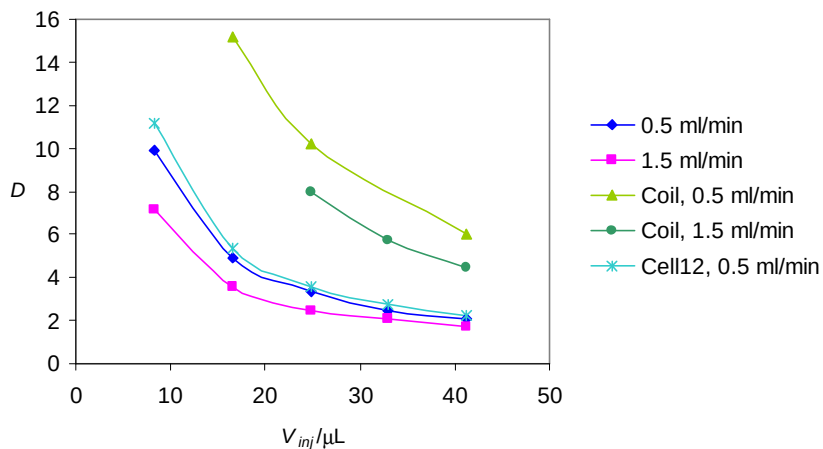


Figure 5. Dispersion coefficients obtained for different dye-injection volumes at main-stream flow rates of 1.5 and 0.5 ml/min in the range of the red light. The experiments were performed with the 10a spectrometric glass coil-type flow-through cell and no mixing coil, if not stated differently.

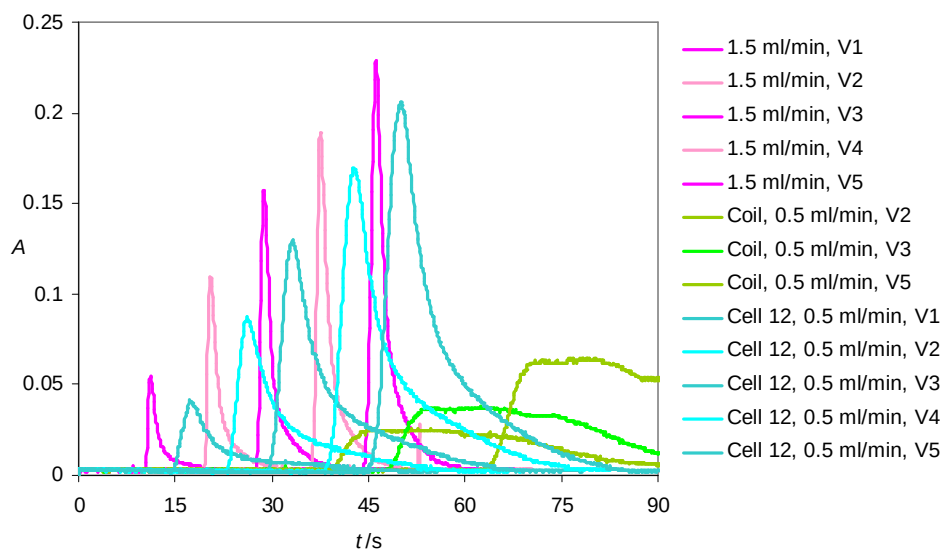


Figure 6. FIAGrams obtained for different dye-injection volumes 8.3 μL (V1), 16.5 μL (V2), 24.8 μL (V3), 33.0 μL (V4) and 41.3 μL (V5), under selected experimental conditions as specified in the legend.

The results, which are presented in Figure 5, proved that even though in its present form the FIA system with 1-mL syringe pumps has a maximum flow rate limited to 1.5 mL/min it allows for a wide variety of dispersion coefficients in the range of medium ($D = 2\text{--}10$) and large dispersions ($D > 10$). The shapes of the peaks that were obtained for different dye-injection volumes, 8.3, 16.5, 24.8, 33.0 and 41.3 μL (V1-V5), under selected experimental conditions are presented in Figure 6. The peaks in magenta were obtained for the experimental series with the lowest dispersion (1.5 mL/min, 10a cell, no

mixing coil), the peaks in green were obtained for the experimental series with the highest dispersion (0.5 mL/min, 10a cell, mixing coil, tubing length 35.2 cm) and the peaks in blue were obtained for the experimental series with moderate dispersion (0.5 mL/min, cell 12, no mixing coil). The shape of the peaks promises that low-cost data-acquisition devices with a limited data-point sampling rate are satisfactory.

In order to prove this presumption and to further extend the experimental opportunities for the low-cost FIA, the set-up was rearranged as shown in Figure 7, so that it allows for volume-based injection. The injection valve assembling procedure is described in the Experimental section 3.2. A low-cost counterpart (€180) which was used successfully as an interface and as a data acquisition device for the spectrometric detector is the digital multimeter M-3860, (Metex Corporation, Korea) with a serial output and supporting software. The spectrometer's analogue output was connected to the multimeter's voltage input, and its serial output was connected to the computer. The supporting software allowed data acquisition at a maximum rate of 1 measurement per second.

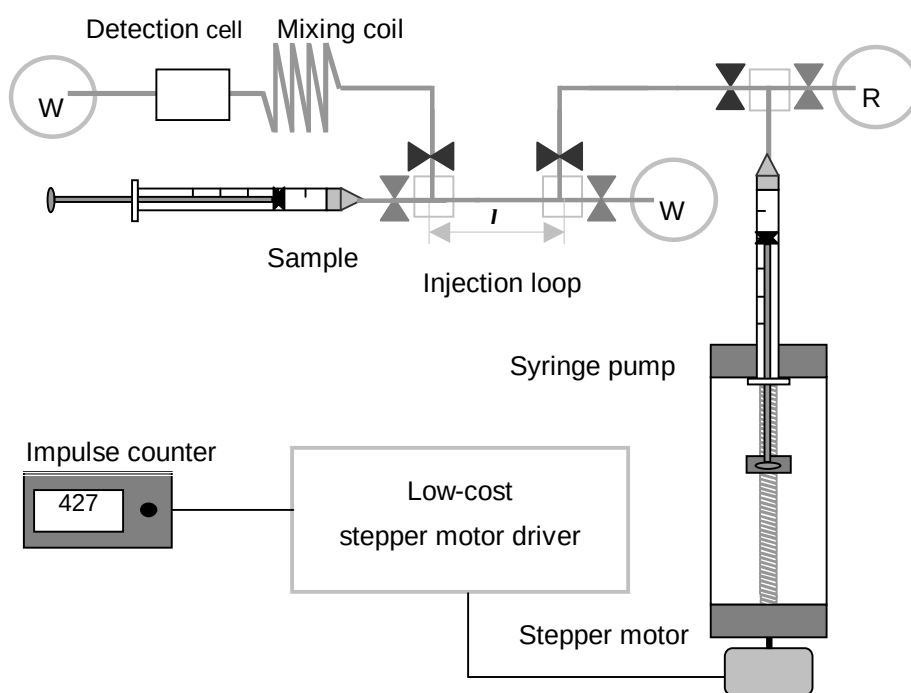


Figure 7. The low-cost simplified FIA set-up with volume-based injection, the assembling of which we suggest for low-budget circumstances and which we used for testing the proposed spectrometric detector for real-life applications in the green light range. We used this set-up together with a low-cost data-acquisition device with a data-point sampling rate limited to 1 measurement per second.

For the determination of dispersion coefficients with the set-up represented in Figure 7, combinations of five injection loops, with volumes in the range between 6.3 and 52 microlitres, and five mixing coils of different shapes with the length of the coil tube ranging from 15.1 to 35.2 cm or no coil at all (T-0xd0) were used. The characteristics of the mixing coils designed from the polymeric tube are given in more detail in the Experimental section 3.2 – Table 3. The dispersion coefficients determined under different experimental conditions at the flow rates 1.5 mL/min and 0.5 mL/min are represented in Figure

8. The low-cost data-acquisition device with a data-point sampling rate limited to 1 measurement per second proved compatible with the proposed FIA set-up and adequate for obtaining useful results.

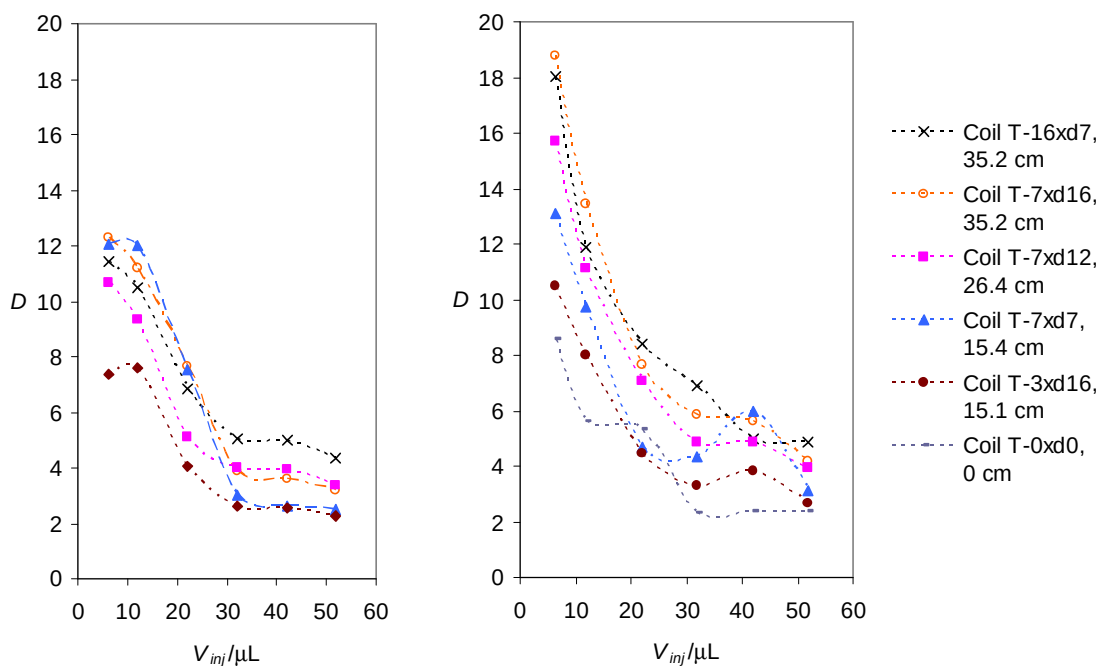


Figure 8. Dispersion coefficients obtained for different dye-injection volumes and mixing-coil lengths and geometries at the flow rates 1.5 ml/min (left) and 0.5 ml/min (right).

The proposed FIA set-ups with the tri-colour LED-based spectrometric detector proved to be fit for the purpose of getting acquainted with the fundamental concepts of FIA and a promising one for simple single-reagent applications with time- or volume-based injection.

Real-life application in the blue light range - Chloride determination

Chloride determination in the concentration range normally expected for drinking water was selected as a test of the suitability of the proposed spectrometric detector for real-life applications. This application was selected because it requires the use of the blue light emitter, which is the weakest light source in the proposed detector, and the adequacy of which is the most questionable, especially for real-life applications where the colour-forming reagent also absorbs light to some extent in the wavelength range of the spectrometric determination as it is with the chloride determination. The composition of the colour-forming reagent is specified in the Experimental section 3.3.

The peaks obtained for the calibration solutions (5–70 mg/L) in the flow-system (Figure 4) with a mixing coil (35.7 cm) and the spectrometric detector with the 11a cell are presented in Figure 9/left. The operation of the flow system was divided into the injection and elution periods, regulated by the precision interval switch. With a time-based injection, 29 μL of the calibration solution were introduced into the main line at a flow rate of 56 $\mu\text{L}/\text{min}$. At the moment of the elapsed injection time period, the precession interval switch switched on the main-stream pump, which operated at a flow rate of 1.25 mL/min. The peak heights were related to the chloride concentration, and a smooth polynomial line with

a high correlation coefficient was obtained, as demonstrated in Figure 9/right. This experiment confirmed that the proposed spectrometric detector gives useful results and is sensitive enough for real-life applications in the range of the blue light, which was considered to be the most problematic.

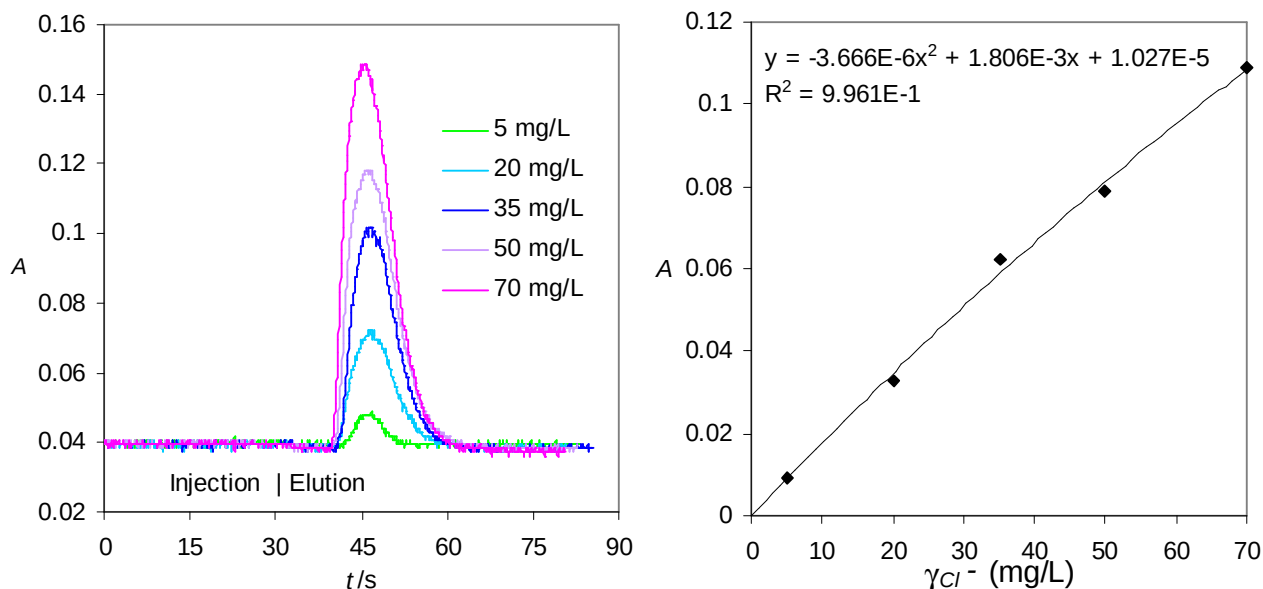


Figure 9. FIAgrams (left) and calibration line (right) obtained for calibration solutions with chloride concentration extending from 5 to 70 mg/L.

Real-life application in the green light range - Calcium determination with a data-point sampling rate limited to one measurement per second

The performance of the low-cost system (Figure 7) with its computerised data-sampling rate limited to one measurement per second was tested for the determination of calcium ions. The experiments were performed at the maximum flow rate of the colour-forming reagent (1.5 ml/min). The composition of the colour-forming reagent is specified in the Experimental section 3.3. The spectrometric flow-through cell with an internal volume of 41 microlitre and a 32-microlitre injection loop were used with two different mixing coils: a 35.2-cm coil T-7xd16 and a 15.4-cm coil T-7xd7. A green LED was used for the spectrometric measurements. The concentrations of calcium ions in the injected solutions ranged from 1 to 100 mg/L. With the longer mixing coil the absorbance starts to increase after 10 seconds and reaches the baseline again after 43 seconds; with the shorter mixing coil the times are 6 and 36 seconds, respectively. The peak widths at half maximum are 12 and 10 seconds with the longer and shorter mixing coils, respectively. Taking into account these data and the time needed for syringe refilling and manual operation of the system, very similar estimations of the maximum frequencies for the sample analysis were obtained for both FIA system geometries at 35 and 36 samples per hour.

The peak heights obtained for two repeated sets of measurements in the FIA system with the T-7xd7 coil and the T-7xd16 coil relating to the concentration of calcium ions are presented in Figure 10. Smooth, repeatable curves with a 3rd-order polynomial trend line as a good fit were obtained. The correlation coefficients (r) were high: between 0.9984 and 0.9994. The shorter mixing coil (T-7xd7) gave a higher sensitivity. A more in-depth analysis of the results proved the linear relation between the

peak height and calcium-ion concentration up to 40 mg/L. The resulting equations of the calibration lines are as follows: $y = 1.013 \cdot 10^{-2} x - 1.562 \cdot 10^{-2}$ ($r = 0.9981$) and $y = 9.911 \cdot 10^{-3} x - 1.570 \cdot 10^{-2}$ ($r = 0.9980$) for the set up with the higher sensitivity and $y = 8.493 \cdot 10^{-3} x - 1.123 \cdot 10^{-2}$ ($r = 0.9989$) and $y = 8.255 \cdot 10^{-3} x - 8.510 \cdot 10^{-3}$ ($r = 0.9979$) for the set up with the lower sensitivity.

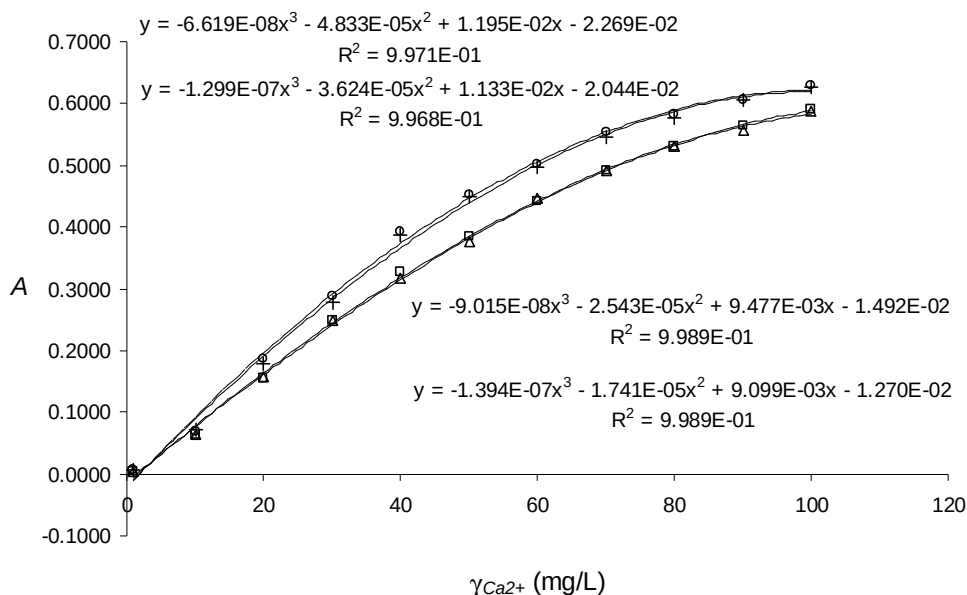


Figure 10. Calibration graphs for calcium ions obtained for two repeated sets of measurements in the FIA system with the T-7xd7 coil or T-7xd16 coil.

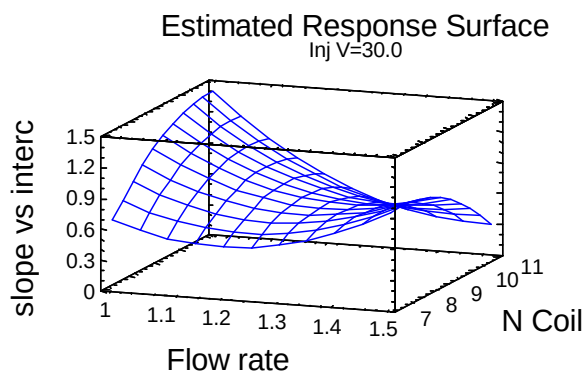
Real-life application in the red light range – Hydrogen phosphate determination with a data-point sampling rate limited to one measurement per second

The performance of the low-cost system in the red light range was tested for a determination of hydrogen phosphate. A similar set-up as for the determination of chloride (Figure 4) was used, but with a different experimental approach and with the computerised data-sampling rate limited to one measurement per second. A 1-mL syringe of the main stream pump was replaced with a 5-ml syringe, and used to propel the hydrogen phosphate solution through the main line. With a time-based injection the Malachite Green – heptamolybdate colour-forming reagent was introduced into the main line at a flow rate 75 $\mu\text{L}/\text{min}$. The composition of the colour-forming reagent is specified in the Experimental section 3.3. In the preliminary experiments high intercept values and low slopes of the calibration lines were observed for the hydrogen phosphate concentration range 0.1–1.3 mg/L. In order to obtain a calibration line with maximal slope and minimal intercept value an optimization of the main stream flow rate, the number of turns of the mixing coil with a coil support diameter 7 mm and the reagent injection volume was performed following the randomized Box-Behnken – response surface design, as specified in Table 2. The experiments were performed with the 0.4 and 1.0 mg/L hydrogen phosphate solutions.

Table 2. Box-Behnken – response surface design for hydrogen phosphate determination optimization.

Experiment	Flow rate (mL/min)	Number of turns of a mixing coil	Injection volume (μ L)	Response variables		
				Slope b	Intercept a	b/a
1	1.5	9	25	0.1197	0.0823	1.4534
2	1.25	11	35	0.1183	0.1445	0.8191
3	1	9	25	0.1532	0.0955	1.6033
4	1.25	9	30	0.1043	0.1368	0.7629
5	1.25	7	35	0.1253	0.1343	0.9335
6	1.25	9	30	0.1268	0.1424	0.8909
7	1.25	7	25	0.0303	0.2389	0.1270
8	1	9	35	0.1592	0.1524	1.0442
9	1	11	30	0.1618	0.1197	1.3524
10	1.5	11	30	0.0387	0.1956	0.1976
11	1.25	9	30	0.0880	0.1600	0.5500
12	1.25	11	25	0.0847	0.1173	0.7216
13	1.5	7	30	0.1272	0.1269	1.0018
14	1	7	30	0.1102	0.1370	0.8039
15	1.5	9	35	0.0860	0.1760	0.4886

The Statgraphics Centurion - version XV software (Statpoint, Inc, Herndon, USA) was used for the experimental design selection and data analysis. The estimated response surface for the slope *versus* intercept quotient (b/a) at the 30 μ L injection volume of the colour-forming reagent is shown in Figure 11. The optimal experimental conditions for the maximised slope *versus* intercept quotient that were recognised by the Statgraphics Centurion software are as follows: the 1.0 mL/min main stream flow rate, the mixing coil with 11 turns and the injection volume of the colour-forming reagent 25 μ L. The same experimental conditions were suggested for the minimization of the intercept value. In spite of the fact that the optimum lies in the corner of the experimental design the prediction was recognised as an optimal solution. Subsequently lowering the flow rate and extending the mixing coil length might have resulted in an even higher slope *versus* intercept quotient, but it would also inevitably extend the peak elution time.

**Figure 11.** The estimated response surface for the slope *versus* intercept quotient (b/a) at the 30- μ L injection volume of the colour-forming reagent.

The equations of the fitted model calculated by the Statgraphics Centurion software were used to predict the slope and intercept of the hydrogen phosphate calibration line under the optimal experimental conditions; the calculated values were 0.167 and 0.0718 respectively. Experiments were performed under the predicted experimental conditions. The peaks and calibration line obtained for the hydrogen phosphate calibration solutions 0.1–1.3 mg/L are presented in Figure 12. The hydrogen phosphate concentration determination with the proposed FIA set-up and time-based colour forming reagent injection with low 25- μ L reagent consumption per determination proved possible.

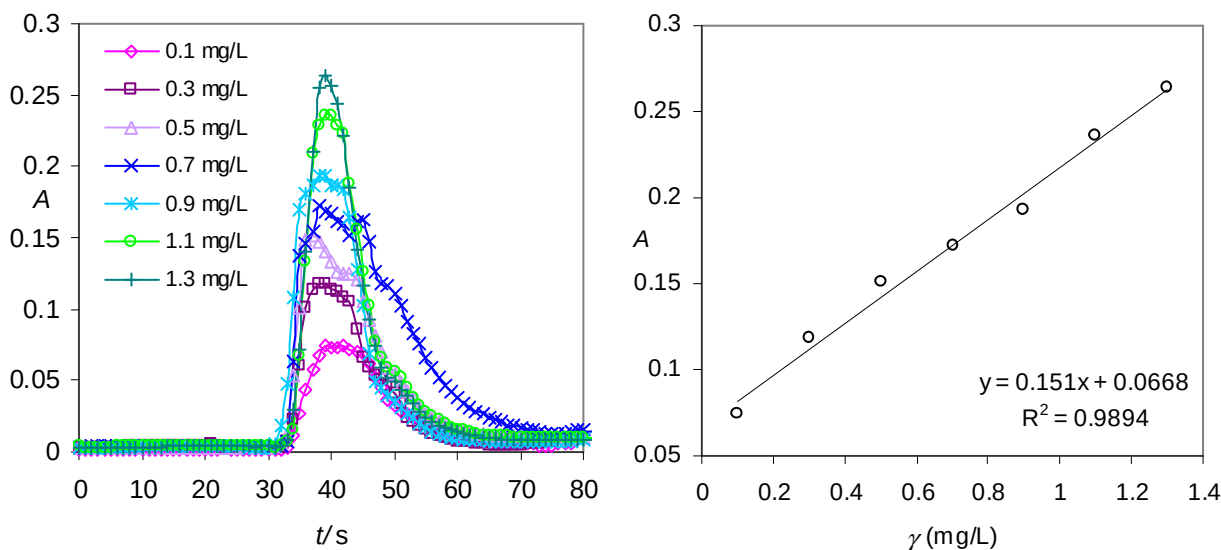


Figure 12. FIAgrams (left) and calibration line (right) obtained for calibration solutions with hydrogen phosphate concentration extending from 0.1 to 1.3 mg/L.

3. Experimental Section

3.1. Prototyping of a tri-colour LED-based detector

Optical geometry

The optical geometry of the spectrometric detector is presented in Figure 1. The glass coil-type flow-through cells were designed from glass capillaries with an OD = 1.35 mm and an ID = 0.95 mm, which were purchased from Euroglass d.o.o. (Ljubljana, Slovenia). The capillaries were shaped into the form of a coil in a propane-butane flame. The coil-type flow-through cell starts with a glass tube inlet section in the horizontal plain. The tube then turns perpendicularly and goes vertically to the bottom of the measuring seat, where the coil with up to four ascending turns begins. The coil ends with the outlet tube section, which lies in the same plane as the inlet tube section. Coils with different outer diameters and consequently different internal volumes were produced, but they were all approximately eight millimetres high, to fit into the same measuring seat, at the bottom of which lies a photoresistor. The cells were assigned the labels 10a, 11a and 12. They were shielded so that when they were filled with deionised water and their positions between the tri-colour LED and the photoresistor were carefully

adjusted with small displacements in the horizontal direction, the light of any of the light emitters – red, green or blue – passed vertically through the coil in its axial direction. The light emitted by the LED was prevented from not passing through the coil by a black filling inside the coil and by blinding shields on the top of the coil and over the bottom of the measuring seat. The best performance was achieved if a black sleeve was also put around the coil so that only the lowest turn of the coil remained free.

Electronic circuitry

The electronic circuitry for powering the 5-mm white-diffused tri-colour LED (Kingbright®) and the photometric detection system arrangement based on a photoresistor (R_x) are schematically represented in Figure 15 (left/right). The design containing an unreference LED source and the signal linearly related to the transmittance was used. The light intensity of the emitters of red (R-LED), green (G-LED) and blue light (B-LED) can be regulated separately by using related potentiometers. In our studies the analogue output of the detector was basically acquired by a computer and by National Instruments LabView software. An appropriate virtual instrument was designed. The output is offset by a manually operated potentiometer. It should be stressed that due to the relatively wide peaks obtained with the proposed FIA system the requirements for the data-sampling rate are not high and several low-cost solutions for data registration or acquisition would do equally as well. The low-cost solution (€180) we used was a digital multimeter M-3860M, (Metex Corporation, Korea) with a serial output and supporting software.

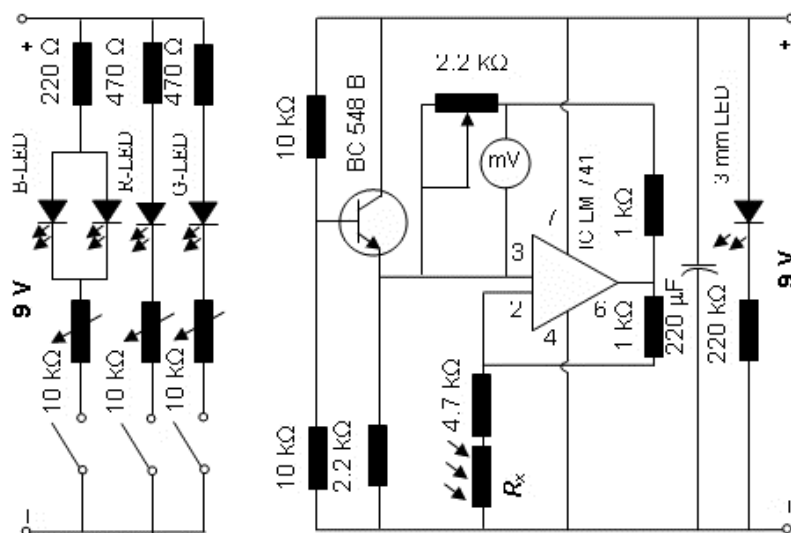


Figure 13. Electronic circuitry for powering the tri-colour LED (left) and the photometric detection system (right).

3.2. Prototyping of a low-cost FIA with time-based injection

The low-cost FIA set-up is schematically represented in Figure 4. It consists of two laboratory-made syringe pumps – a main-stream pump and an injection pump – with related drivers wired to the precision interval switch, which enables time-based injection.

Each pump consists of a 1-mL syringe, a syringe holder and a unipolar 12-V stepper motor (Premotec 9904 11235014, Holland), which was purchased from Bürklin (Düsseldorf, Germany). Typical controllers for unipolar stepper motors are based on a combination of switches and a control unit, which is responsible for providing the control signals to open and close the switches at the appropriate times in order to spin the motor. The control unit is usually a computer or a programmable interface controller, with software directly generating the outputs needed to control the switches. Low-cost stepper-motor drivers based on an SN74LS194 bidirectional universal shift register were assembled instead; the driver-circuit schemes, which are available on the web, were used as a starting point [13]. In order to make the low-cost stepper-motor driver fit for the purpose we introduced some necessary adjustments.

The driver enables the basic switch-operated control functions – forward, reverse, stop – and a potentiometer-settable speed adjustment. The clock output of the driver to which an impulse counter (Type CUB III, Wachendorff, Germany) was connected was used to control the number of steps performed by the motor. Taking into account the syringe's geometry and the elapsed time made it possible to calculate the pump's flow rate. One turn of the motor consisted of 48 steps, causing a 1-mm displacement of the syringe's piston. The operation of the syringe pump was limited by two microswitches, which automatically switched off the stepper-motor driver if the syringe piston reached any of its end-stop positions. These relate to the syringe being either entirely full or nearly empty, and so prevented damage to the system.

Components for the precision interval switch (Order number 191299) were purchased from Conrad Electronic (Wernberg-Köblitz, Germany) and soldered in accordance with the electronic scheme of the producer; however, a modification was introduced so that two 500-k Ω potentiometers regulating the time intervals of the normally closed and normally open relay outputs were replaced with 15-segment resistor cascades of the same overall resistance, which allowed for fifteen different but repeatable timings of both relay outputs. The main-stream pump and the injection pump were connected to the normally closed and the normally open relay connectors, respectively. Fifteen precisely defined time intervals between 0.3 and 44.3 seconds could be selected for both relay outputs. A precision time switch (Order number 191280) can be used instead.

Polymeric tubing (C-FLEX® Tubing (ID 0.031, OD 0.094), Cole-Parmer Instrument Company, Illinois) was used for the liquid lines. Disposable syringe needles (PrecisionGlide® Needle 18G1 $\frac{1}{2}$, Becton Dickinson and Company Franklin Lakes, New Jersey) were used in different ways: either a needle with a cut-off tip and a polished remaining end was used to connect the syringe to a tube, or needle pieces cut to an appropriate length and with the ends polished were used as tubing connectors or in the design of the valves.

The valves for redirecting the liquid streams were produced from 8-mm-thick Plexiglas plate, cut into chips with a rectangular shape and sides of 10 mm. Holes were drilled from the sides using a 1.2-mm (OD) drill bit so that T-shaped channels were produced. The external connectors were designed so that the pieces of the syringe needles were screwed tightly into the channels, as close to the intersection of the channels as possible. Polymeric tubes were attached to the loose ends. The valves functioned by manually clamping the appropriate tubes at the outlets of the valves using paper clamps, schematically indicated in Figure 4 with two opposing triangles showing the possible positions of the paper clamp.

The two alternative positions of the clamp, relating to the two operational phases of the valve, are indicated in different colours – dark grey and light grey. A liquid line that is currently clamped is closed.

The injection valve consists of two T-pieces, connected either rigidly with one longer piece of a syringe needle from the original metallic tubing, or for larger volumes, two T-pieces were connected with the polymeric tubing used for the liquid lines. Several injection valves with different loop volumes were produced with 6.3, 12, 22, 32, 42 and 52 microlitre injection loops.

The mixing coils were designed from the polymeric tube and the rigid tubing with a 7-mm, 12-mm or 16-mm diameter. Coils with different diameters and with different numbers of turns and different coil-tubing lengths were produced, as specified in Table 3. The coil's inlet and outlet connection tubing were 6 and 4 cm long, respectively. It was designed so that two holes were drilled into the coil support tubing on the same sides of the tubing, and at a distance required by the polymeric tube coil in between, so that the coil's inlet and outlet tubing passed smoothly through the holes to the inside of the support tubing, preventing the coil from unwrapping or becoming loose. The coil's form was further secured with transparent adhesive tape.

Table 3. Characteristics of mixing coils designed from the polymeric tube.

Mixing coil's mark	T-16xd7	T-7xd16	T-7xd12	T-7xd7	T-3xd16	T-0xd0
Number of turns	16	7	7	7	3	-
Coil support diameter	7 mm	16 mm	12 mm	7 mm	16 mm	-
Coil tube length*	35.2 cm	35.2 cm	26.4 cm	15.4	15.1 cm	-

*Each coil's inlet and outlet connection tubing which was six and four centimetres, respectively was not taken into account in the coil-tube length.

3.3 Performance tests

Substances with absorption spectra that effectively overlap with the emission spectra of the light emitters had to be selected for the detector's performance test. A 20-mmol/L ammonium acetate solution containing 3.5% (v/v) of Mint natural aroma and 3.5% (v/v) of Pear aroma (both purchased from Tovarna eteričnih olj in pijač, Slovenia) proved a suitable combination of food dyes, E102, E110 and E131, which gave a spectrum with two wide, well-separated absorption peaks of similar height with absorption maxima at 418 and 662 nm, respectively. For this reason this stock solution (Solution I) was used for the detector's performance test, i.e., a linearity and repeatability test in the range of the blue and red light and additionally for determining the dispersion coefficients for the low-cost FIA set-up. Prior to preparing the working solutions by further diluting the stock Solution I with 20-mmol/L ammonium acetate solution, n-hexane extractable substances were removed from the Solution I. The spectrometric measurements were made against the 20-mmol/L ammonium acetate solution as a blank.

For the detector's performance evaluation in the range of green light a 3-mmol/L stock solution of potassium manganate(VII) (Solution II) was prepared with deionised water. The working solutions

were prepared from Solution II with further dilutions using deionised water, which also served as a blank for the spectrometric measurements.

The suitability of the proposed detector for real-life applications was tested on an example of chloride determination in the concentration range that is normally expected for drinking water. For the preparation of the colour-forming reagent $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, $\text{Hg}(\text{SCN})_2$, HNO_3 ($w=0.65$, $\rho=1.4$ kg/L) and methoxy-2-ethanol ($\rho=0.96$ kg/L), which were all *p.a.* grade chemicals purchased from Merck (Darmstadt, Germany), were used. A solution of $\text{Hg}(\text{SCN})_2$ was prepared in a dry 20-ml volumetric flask from 1.908 g of chemical, which was dissolved and diluted with methoxy-2-ethanol. The colour-forming reagent was prepared in a 500-ml volumetric flask into which 10.1 g of $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ was quantitatively transferred and dissolved with the required quantity of deionised water. A 3.3-ml addition of the previously prepared $\text{Hg}(\text{SCN})_2$ solution and a 1.7-ml addition of concentrated HNO_3 ($w=0.65$, $\rho=1.4$ kg/L) followed successively. This solution, subsequently referred to as Solution III, was finally diluted to the mark with deionised water. Calibration solutions were prepared with deionised water from NaCl of *p.a.* grade, which was purchased from Kemika (Zagreb, Croatia).

The reagent for determining the amount of calcium was prepared from 0.0187 g of Phtalein purple (o-cresolphthalein complexon, $\text{C}_{32}\text{H}_{32}\text{N}_2\text{O}_{12} \cdot x\text{H}_2\text{O}$, $M = 636.62$ g mol⁻¹, Merck, Darmstadt, Germany), 0.0259 g of 8-hydroxyquinoline ($\text{C}_9\text{H}_7\text{NO} \cdot 1/2\text{H}_2\text{SO}_4$, $M = 194.2$ g mol⁻¹, Sigma, St. Louis, USA), 0.193 g of NaHCO_3 and 0.816 g of Na_2CO_3 dissolved consecutively and diluted with deionised water in a 100-mL volumetric flask [14]. Calibration solutions with calcium-ion concentrations ranging from 1 to 100 mg/L were prepared from a stock calcium chloride solution with a calcium-ion concentration of 1 g/L.

The reagent for determining the amount of hydrogen phosphate [15] was prepared from 30 mL of the stock ammonium heptamolybdate solution, 25 mL of the stock Malachite Green solution and 4.7 mL of concentrated sulphuric acid. After allowing the mixture to stand for 30 min, the solution was filtered through a 0.45- μm membrane filter-paper. The stock ammonium heptamolybdate solution was prepared by dissolving 6 g of $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ ($M = 1235.86$ g/mol, Carlo Erba, Milan, Italy) in 50 mL of deionised water. The stock Malachite Green solution was prepared by dissolving 0.244 g of Malachite Green oxalate ($(\text{C}_{23}\text{H}_{25}\text{N}_2)_2 \cdot 3\text{C}_2\text{H}_2\text{O}_4$, $M = 929.0$ g/mol, Sigma, St. Louis, USA) in 250 mL of deionised water. Calibration solutions were prepared from a stock disodium hydrogen phosphate solution with a hydrogen phosphate-ion concentration of 1 g/L.

Conclusions

The novel optical geometry of the proposed empirical spectrometric detector proved to be fit for the purpose for which it was constructed and useful for real-life applications. Measurements in three spectral ranges are possible with a minimal number of optical components, including only a light source and a sensor. The detector is a small, compact, low-energy-consumption device that can be easily assembled for less than €80. The proposed spectrometric detector together with the low-cost simplified FIA, which we additionally proposed and tested, and the assembling of which is possible for approximately €625, gives schools with a limited budget the chance to offer their students a hands-on experience with FIA from which they can benefit a great deal.

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