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A new type of a spectrometric microtitration set up

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Abstract

A spectrometric microtitrator was developed from a spectrometer with a microreaction chamber and a tri-colour light-emitting diode (LED) as the light source. A novel, vertical, optical geometry of the spectrometric microtitration chamber was introduced and tested. This novel geometry also required a new method for mixing the titrated solution. A laboratory-made 50 μl syringe pump was used for the addition of the titration reagent. The 10-channel module for light effects, which makes possible a low-cost hardware approach to changing the titration protocols, was used for coordinating the operation of the microtitration set up. The system, with 10 channels and a regulated speed of operation, is flexible enough to allow an operator to generate different titration protocols. The performance test showed that the speed of titration-reagent addition can be regulated in the range from 0.87 to 21.8 μl min^{-1}. The smallest achievable volume addition is equal to 35 nl. The mixing rate can be continuously regulated by an electrical pulse that initiates the mixing cycle. The quickest rate is every 1.6 s, and the slowest rate is every 4.8 s. The spectrometric microtitration set up was successfully tested for several different real-life spectrometric titrations, including an iodometric titration, a determination of CO_2 in deionised water, and EDTA titrations of copper(II) ions with no indicator. The volume of the examined solution can be as small as 220 μl. The titration-reagent consumption is usually between 10 and 35 μl. Coefficients of variation of the end point volume determination ($n = 5$) at different experimental conditions and different average volumes of consumed reagents (7.06, 12.17 and 22.88 μl) were 2.4, 1.3 and 1.2%, respectively. The novel geometry of the spectrometric microtitration chamber proved to be useful for real-life applications.

Keywords: Spectrometric microtitrator; Light emitting diode; Alkalimetric titration; Iodometric titration; EDTA titration

1. Introduction

Even though it is an old technique, titration remains the method of choice for the determination of several macro-components in real samples. Various techniques can be used to detect the end-point of the titration, one such technique is spectrometric detection. In 1973 Flaschka et al. [1] proposed a spectrometer based on light-emitting diodes (LEDs). Since then LEDs have frequently been used as light sources in spectrometric detection for a range of applications. LEDs, with their small size and low energy consumption, have also brought new opportunities for titration devices based on spectrometric detection. An extensive and concise overview of LED-based detectors from the past decade was recently published by Dasgupta et al. [2].

After 1975, when Ruzicka and Hansen introduced flow-injection analysis (FIA) [3], the foundations were laid for flow-injection titrations, which are now well established [4] and widely used. Although FIA has several advantages, there are also a few drawbacks, e.g., a high consumption of reagent and a lack of robustness. However, some of these problems were overcome by the introduction of sequential injection analysis (SIA) [5–7]. Recently, the monosegmented flow approach together with sample-position identification [8] was demonstrated. This enables a complete titration in a single monosegment of the sample, and thus meets the IUPAC definition for a titration in all aspects, even though it is made in a flow system.

In real life batch titrations dominate totally, even though the daily users of batch titrations do not publish results so often. Titrations based on the flow-injection principle are used by a very limited number of practically working analytical chemists. An early description of a microbatch analyser, in

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which a combination of an LED and a photodiode was used for the spectrometric detection of the titration's end-point, was given in 1988 by Sweileh and Dasgupta [9]. Two plastic optical fibres are inserted in the cylindrical wall of the reaction chamber, on opposite sides, such that the ends of the two fibres are exactly opposite and flush with the chamber wall. The ends of the fibres are coupled to an LED and a photodiode. Such as setup was successfully used for three different acid-base titrations.

Tan and co-workers, who recently suggested several different microtitration methods with LED-based spectrometric detection [10–12], used a self-constructed titration cell with an optical geometry very similar to that of Sweileh and Dasgupta. Near the bottom of a titration cell, which is made of glass, are mounted an LED and a phototransistor. The phototransistor is opposite the LED at a distance of 10 mm. A magnetic stirrer is used for the rapid homogenisation of the solution. The automation of the sampling and titration procedure is based on a 16-port rotary valve and linear-flow piston pumps with disposable plastic syringes of 10 ml, which are operated with a single-chip microcomputer.

In our previous paper we described a tri-colour LED-photoresistor-based in situ spectrometer with a geometry that differs from those described above and is suitable for small volumes of test solutions (350 μl) and the effective homogenisation of reagents [13]. Polymeric supports, called blisters – used in the pharmaceutical industry for the packaging of pastilles – function as the reaction and measuring chambers. Each support has five pairs of concave hollows. The measuring chamber is designed so that the light emitted by an LED passes through the solution in the vertical direction and falls directly on a photoresistor, which lies under the hollow of a blister, in close proximity to the bottom of the hollow.

Here, we present a spectrometric microtitrator that was developed from a spectrometer with a microreaction chamber and a tri-colour LED as the light source. The novel geometry of the spectrometric microtitration chamber demanded a new approach to the mixing of the titrated solution. A low-cost hardware approach to changing titration protocols is presented and used for testing the novel geometry of the spectrometric microtitration chamber. The prototype was tested for some real-life titrations of various types.

2. Experimental

2.1. The transformation of a spectrometer with a microreaction chamber into a spectrometric microtitrator

The specific geometry of the spectrometer, which is described in more detail elsewhere [13], involves the light of a tri-colour LED passing through the solution in the vertical direction and falling directly on a photo-resistor, which lies under the concave microreaction chamber. In order to transform the microreaction chamber into a microtitration chamber, it was necessary to add the titration reagent and mix the titration solution.

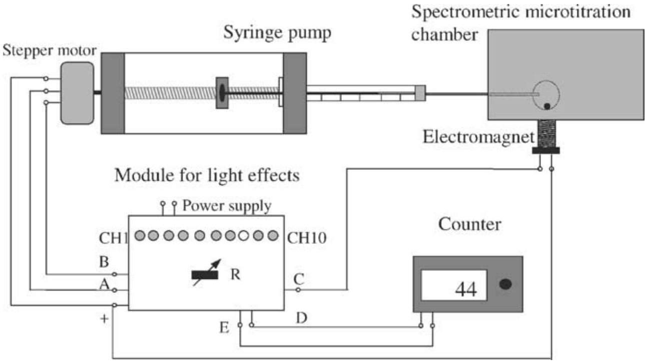

Fig. 1. Spectrometric microtitration set up.

The microtitration set up is schematically represented in Fig. 1. A laboratory-made syringe pump, driven by a stepper motor, was designed to add the reagent to the microtitration chamber. The pump consists of a Hamilton syringe (50 μl), a syringe holder and a unipolar stepper motor, which was purchased from Bürklin (Düsseldorf, Germany). Even though the shape and the size of the hollow of a blister contribute to a rapid homogenisation of the reactants, additional mixing of the solution is necessary for the titration. However, because of the specific geometry of the spectrometer the usual way of mixing, in which a magnetic bar rotates or circulates on the bottom of the reaction vessel under the influence of a permanent magnet rotating below, was not applicable.

A novel approach to mixing was needed, one which would also make it easy to remove the mixing object from the optical path during the measuring process. A bead with a ferromagnetic core and a diameter of between 2 and 3 mm was selected as the mixing object. The mixing process is induced by an electromagnet positioned at the side of the microreaction chamber and fed with appropriate electrical pulses. First, the electromagnet attracts the bead to the side of the hollow of the blister. Next, as the pulse fades the bead starts to fall back into the hollow of the blister under the force of gravity. Repeating the cycle causes the bead to "swing", and this ensures that the solution is mixed. During measurements the bead is attracted to the side of the chamber by the electromagnet and thus remains out of the optical path for as long as necessary.

For the operation of the microtitration set up, a pulse generator for mixing the solution and an appropriate stepper-motor driver were required. 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 programmable interface controller, with software directly generating the outputs needed to control the switches. Our objective was to find a replacement that would make possi-

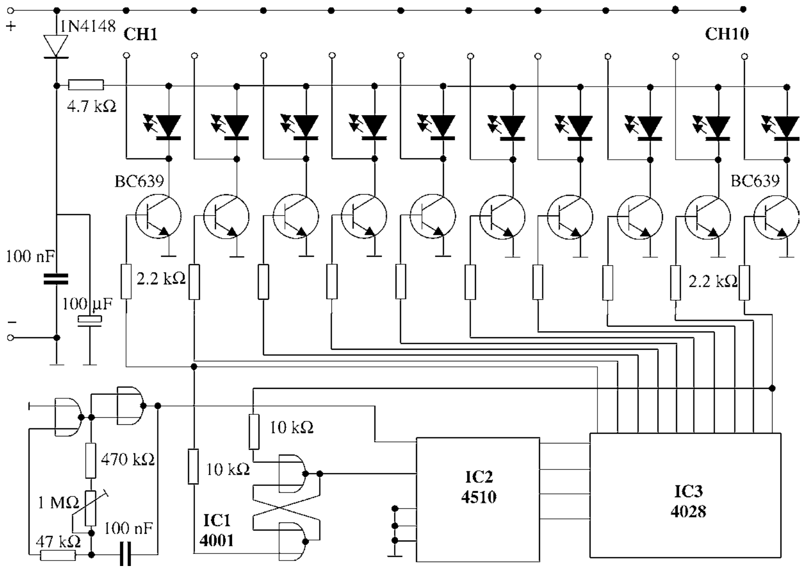

Fig. 2. Module for light effects (Conrad Electronic) with its 10 signal outputs (CH1–CH10), on which the hardware approach to changing titration protocols was based.

ble the low-cost operation of the microtitrator for testing the geometry of the microtitration chamber.

2.2. *Low-cost hardware approach to changing titration protocols*

The solution was a 10-channel module for light effects, purchased from Conrad Electronic (Wernberg-Köblitz, Germany), which would allow us to coordinate the operation of individual parts of the microtitration set up. The module is schematically represented in Fig. 1; a more detailed electronic scheme is shown in Fig. 2. The module operates in such a way that its 10 output channels (CHs) are serially and regularly fed with electrical pulses of 12 V. The sequence starts in one direction, e.g., from CH1 to CH10, and than proceeds continuously and repeatedly in the reverse direction. A variable resistor *R* on the module makes it possible to regulate the speed of operation.

The two signal inputs of the stepper motor (connectors A and B) and the electromagnet (connector C) can be connected to the module in several different ways. A counter is added to register the number of cycles (connectors E and D). The system with 10 channels and a regulated speed of operation is flexible enough to allow an operator to generate different titration protocols. The module for light effects, which provides a low-cost hardware approach to changing the titration protocols, functions as a simplified substitute for the microprocessor used in commercially available titrators.

In the hardware approach to changing the titration protocols the selection of the actual step of a titration procedure is made possible by the combination of the positions of the two switches (not represented in Fig. 1), with each of the two switches having three positions. The positions of the first switch are as follows: the module for light effects ON; the module for light effects OFF; power supply directly wired to the electromagnet. The positions of the second switch are as follows: pulses to motor and electromagnet; STAND BY position; pulses to electromagnet only.

The positions of the two switches make it possible to choose between the three basic modes of operation of the microtitration device. In the first mode the reagent is added in small doses, the solution is stirred, and the counter registers the added volume; in the second mode the solution is only stirred to allow the reactants to react fully, and the counter registers the waiting time; in the third mode of operation the measurement takes place, and the stirring bead is held at the side of the hole of a blister, out of the optical path. The hardware approach to changing titration protocols is easy to apply, and the control over the operation of the microtitration set up is straightforward. In contrast with software-operated systems, no extra time is needed here for familiarization with an algorithm, its parameters and variables, which are usually specific to a particular producer.

2.3. *Performance test*

The performance test was used to determine the working ranges for the fundamental titration parameters, e.g., the speed of reagent addition, the minimum volume increment and the mixing rate.

The speed of reagent addition is affected by the wiring of the motor signal inputs to the module for light effects and by its speed of operation. The slowest, uniform addition of titration reagent, with a speed of 0.87 $\mu l\,min^{-1}$, is achieved with the potentiometer P1 in the far-right position and the stepper motor connected to the channels CH1 and CH10.

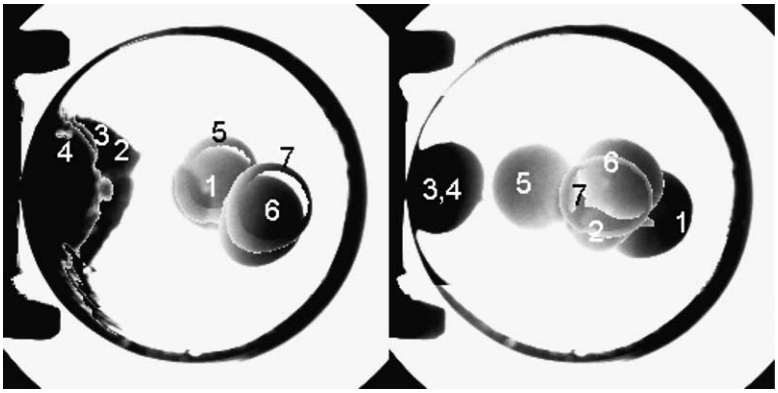

Fig. 3. A novel approach to mixing: the movement of a ferromagnetic mixing bead in the hollow of a blister, imaged with a microscope during two successive mixing cycles (left/right).

For the fastest speed of the titration-reagent addition, the two signal motor contacts are alternatively connected to all 10 channels: one to the odd channels and the other to the even channels. In this case, the potentiometer is in the far-left position. Under these conditions, 21.8 μl of titration reagent are dispensed every minute. With a 50 μl syringe, one turn of the motor corresponds to 0.833 μl of dispensed liquid. Under these conditions the lowest achievable volume increment is 35 nl.

The mixing rate can be continuously regulated by an electrical pulse that initiates the mixing cycle. The quickest rate is every 1.6 s, and the slowest rate is every 4.8 s. The pulse frequency does not, however, tell everything about the mixing process, because the ferromagnetic bead keeps moving even after the electrical pulse has vanished. Therefore, in order to investigate the mixing process in more detail, the movement of the ferromagnetic bead was followed using a microscope, and a sequence of pictures was taken and overlaid. The results are presented in Fig. 3.

Fig. 3 shows the movement of the ferromagnetic bead in the hollow of a blister during two mixing cycles that were initiated by two successive electric pulses. In each picture, number 1 is assigned to the standstill starting position and number 7 to the standstill ending position. In order to clarify some of the overlaid positions of the ferromagnetic bead, some pictures of the bead were partially emptied. The visualisation of the mixing process indicated that the ending position of the bead in the second mixing period (number 7 on the right) matches very closely to its starting position in the previous sequence (number 1 on the left).

2.4. Procedures

Several different titrations, including oxidation-reduction, acid-base and complexometric titrations, were selected to test the usability of the titration set up for real-world applications. The titrations were chosen so that all three light emitters of a tri-colour LED (red, green and blue) played a part. The procedures are as follows.

An aqueous solution of potassium iodate was transferred to a blister, and the solid potassium iodide and 200 μl of HCl solution (0.06 mol l^{-1}) were added. The titration was performed with a standard $Na_2S_2O_3$ solution (0.1 mol l^{-1}) and followed with the blue LED.

For the determination of the amount of CO_2 dissolved in deionised water, 220 μl of the water sample was titrated with a standard NaOH solution (0.1 mol l^{-1}). The phenolphthalein indicator and the green LED were used.

Complexometric titrations were performed using a standard Na_2EDTA solution with a concentration of 0.2 mol l^{-1}. The procedure was a direct titration without any indicator. The red LED was used to follow the titration of the copper sulphate solution, to which 230 μl of acetate buffer (pH 5, 0.65 mol l^{-1}) was previously added.

3. Results and discussion

3.1. Spectrometric titrations with the microtitration device

In order to test the novel geometry of the spectrometric microtitration chamber, three different titrations, including oxidation-reduction, acid-base and complexometric titrations, were performed. The titrations were chosen so that all three light emitters of a tri-colour LED (red, green and blue) were employed. The titrations required different titration protocols in terms of the speed of titration-reagent addition, the volume increment, the mixing intensity and the waiting times. Optimum procedures were quickly and easily obtained by using the hardware approach to changing the titration protocols, which was described previously.

The first titration was designed to check the usability of the microtitration set up for a direct titration of iodine with a $Na_2S_2O_3$ solution. Iodine was obtained on a blister as a result of the reaction between the potassium iodate solution and potassium iodide. This titration had to be performed quickly, with no extra waiting time between the additions. The titration

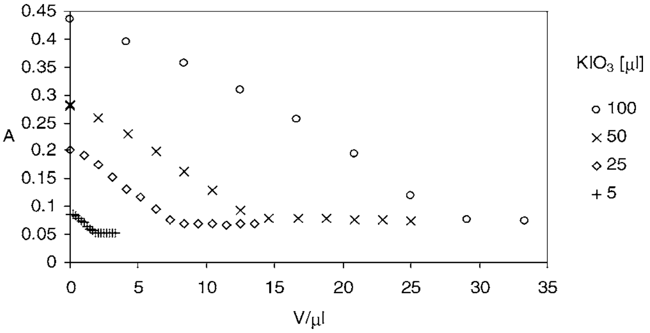

Fig. 4. Spectrometric titration of iodine with the standard $Na_2S_2O_3$ solution (0.1 mol l^{-1}), followed with the blue LED. Different volumes of potassium iodate solution were mixed on a blister with solid potassium iodide. Titration was performed in acidic medium.

curves shown in Fig. 4, proves that the direct titration of iodine with a blue LED is possible. No indicator is needed. Titration was optimised so that similar number of points was obtained for the titration of very different volumes of the potassium iodate solution, ranging from 5 to 100 µl, what demonstrates the flexibility of the microtitration set up. Since iodine can be produced or consumed in reactions with several different species, the experiment indicates the usability of the microtitration set up for iodometric and iodimetric titrations, which are large groups of commonly practised titrations.

This iodometric titration was examined in more details, the relation between the volume of sample solution and the volume of reagent, consumed for the titration was evaluated. The titration protocol was optimised so that minimal volume increments was 0.260 µl. The point density in the resulting titration curves was slightly lower than in the case of a 5 µl sample titration, which is represented in Fig. 4, and for which the titration reagent was added in the 0.208 µl portions. Titrated volumes of potassium iodate solution ranged from 10 to 50 µl. The volume of the reagent consumed for the titration was in all cases calculated at the crossing point of the two extrapolated lines representing the two parts of the titration curve. The last nine points of decreasing part of the curve and first six points of the flat part of the curve were taken into account in all cases. Results are presented in Table 1. A good linearity was observed with the regression line expression $y = 0.2187x + 1.4101$ and correlation coefficient 0.9963.

The titration of a 25 and 50 µl sample was repeated five times, the results are summarised in Table 2. The consumed volumes were determined at the crossing point of the two extrapolated lines representing the two parts of the titration curve, as described previously. Average volumes of consumed titration reagents for titration of the 25 and 50 µl samples were 7.06 and 12.17, respectively. The coefficients of variations were 2.4 and 1.3%.

Table 1
Iodometric titration of different volumes of KIO$_3$ solution with $Na_2S_2O_3$ solution (0.1 mol l^{-1})

Sample volume (µl)	Consumed volume (µl)
10	3.42
20	5.69
30	8.50
40	10.07
50	12.17

Table 2
Repeatability of the iodometric titration

	Sample volume	
	(25 µl)	(50 µl)
	Consumed volume (µl)	
1	7.20	12.02
2	7.15	12.18
3	7.06	12.42
4	7.12	12.06
5	6.77	12.19
Mean	7.06	12.17
s	0.17	0.16
CV	2.4%	1.3%

An acid-base titration was performed next using the green LED with the standard NaOH solution and the phenolphtalein indicator. A titration of carbon dioxide dissolved in deionised water is a titration with a very low titration-reagent consumption. In order to obtain a clearly defined titration curve, additions of the titration reagent need to be sufficiently small. The resulting titration curve, presented in Fig. 5, demonstrates that it is detailed enough.

The final examined titration was complexometric titrations of copper(II) ions with a standard disodium salt-EDTA solution and no indicator. Copper ions produce a complex with EDTA that causes a deep-blue coloration of the solution. Therefore, the titration can be directly followed with the red LED. The titration curves related to titrations of different volumes of examined solutions are presented in Fig. 6.

Since the regent consumption is higher here than in the iodometric titration, the minimal volume increment was increased to 0.55 µl. The consumed volumes were determined at the crossing point of the two extrapolated lines representing the two parts of the titration curve. The procedure was the same as for the iodometric titration. The consumed volumes for the titrations of different volumes of CuSO$_4$ solution are

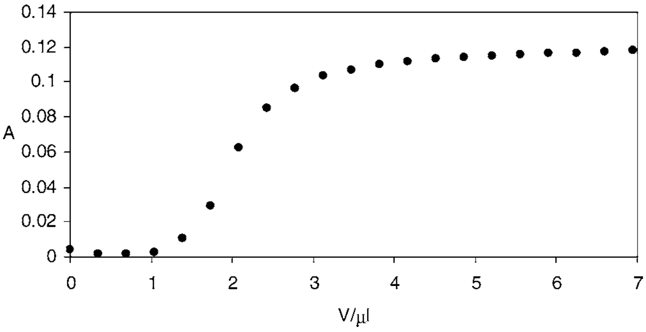

Fig. 5. Titration of carbon dioxide in deionised water. The titration was performed with the standard NaOH solution (0.1 mol l^{-1}) in the presence of the phenolphtalein indicator with the green LED.

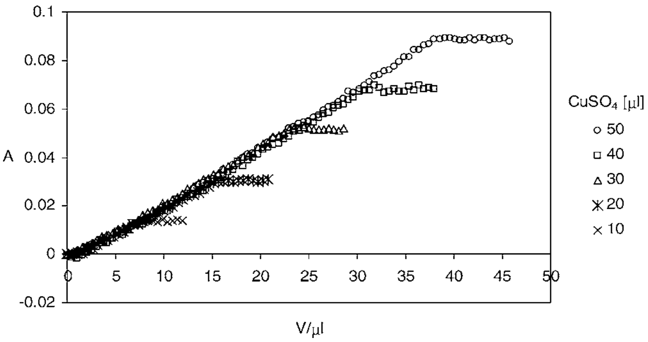

Fig. 6. EDTA titrations of copper(II) ions with no indicator, followed with the red LED. Different volumes of the sample solution were titrated.

Table 3
Complexometric titration of different volumes of $CuSO_4$ solution with Na_2EDTA solution (0.2 mol l^{-1})

Sample volume (µl)	Consumed volume (µl)
10	8.11
20	15.57
30	22.88
40	31.55
50	38.26

Table 4
Repeatability of the complexometric titration

	Sample volume (30 µl)
	Consumed volume (µl)
1	22.64
2	23.15
3	23.07
4	22.52
5	23.04
Mean	22.88
s	0.28
CV	1.2

summarised in Table 3. A good linearity between the sample volume and the consumed volume was observed with the regression line equation $y = 0.7627x + 0.3956$ and the correlation coefficient 0.9994.

The titration of a 30 µl sample was repeated five times, the results are summarised in Table 4. The average volume of consumed titration reagent was 22.88 with the coefficient of variation 1.2%.

4. Conclusions

The spectrometric microtitrator with a novel geometry for the spectrometric microtitration chamber proved to be useful for real-life applications. The new approach to mixing the titrated solution is efficient and reliable. Titration-reagent consumption is small, usually between 10 and 35 µl, and the titration can be successfully performed with a volume of examined solution as small as 220 µl. The hardware approach to changing the titration protocols, which was suggested and used here, was demonstrated to be a useful, low-cost solution for the initial testing of the spectrometric microtitration set up.

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