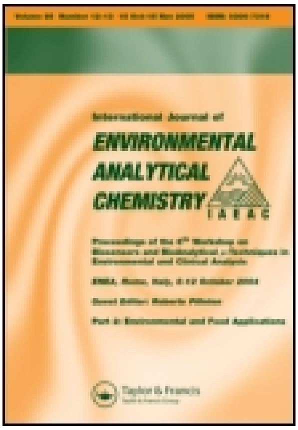

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# Multipumping flow systems devoid of computer control for process and environmental monitoring

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# Multipumping flow systems devoid of computer control for process and environmental monitoring

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In this paper we describe for the first time the use of astable multivibrator circuits for computer-less control of solenoid micropumps and application for analytical flow techniques. Triggering and powering were accomplished using the NE555 integrated circuit. The activation and deactivation time intervals were adjusted using 10-turn trimmer potentiometers. The potential and characteristics of the instrumentation were studied on a two-channel flow system injecting an indigo carmine indicator solution. Subsequently, a three-channel flow system was assembled and successfully applied to the determination of nitrite in well waters. One circuit was used to control the activation time intervals of three further circuits used for the control of the flow rates or pulsation of solenoid micropumps. These were used for driving carrier, reagent, and sample in an analytical flow system. In the present work, the utility of the circuits for the construction of simple multipumping flow systems was demonstrated. A main feature to be highlighted was the simplicity and very low costs of the controlling circuits, favouring economic and miniaturised flow analysers. Second, no expenses or knowledge are required for the usual software control of the solenoid micropumps. This allows working with every existing detector without considering any problems of software and control compatibility. Third, owing to these features, the proposed assembly is especially suited for simple monitoring analysers, sample provision from an environmental or industrial process, or chemical education.

**Keywords:** computer-less control; astable multivibrator; solenoid micropump; multipumping; nitrite

## 1. Introduction

Solenoid micropumps (SMP) are used as economic and simple liquid drivers and as an alternative to peristaltic and syringe pumps in industrial applications such as dosing or providing process streams to analytical instrumentation. One of the first applications of solenoid pumps as liquid drivers in analytical flow techniques (FT) was reported by Weeks and Johnson (1996) [1] and was aimed at the miniaturisation of field applicable FT systems. Up to that date, solenoid actuators presented state-of-art in microflow systems (chips) as overviewed in a technical review two years before [2]. The usefulness of the

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semi-continuous highly pronounced pulsated flow of SMP causing intermediate turbulent conditions and enhanced mixing conditions was first discussed by Lapa *et al.* (2002) [3], coining the term ‘multipumping flow systems’ (MPFS).

To date, SMP have been used in confluent multichannel flow systems as typical for flow injection analysis (FIA) [4] as well as in counter-directional mono-channel sequential injection analysis (SIA) assemblies [5,6]. Comprehensive reviews of MPFS can be found elsewhere [7,8]. In contrast to and in advantage over peristaltic pumps-based flow systems, SMP allow the control of each flow channel individually. However, software control seems to be an obligatory requirement, which is achieved by means of relay cards.

In spite of the commercial availability of economic relay cards for the control of SMP, there is still a need for advanced software control for these devices. Such software solutions can present a considerable contribution to the final expense for the MPFS assembly. Moreover, there are hardly any products commercially available that are capable of accomplishing the control of both solenoid micropumps and possible detectors of interest. Among the few that are available, the software AutoAnalysis [9,10] developed in our research group and distributed by Sciware SL (Palma de Mallorca, Spain) was used in this work for data acquisition and evaluation.

In this paper we describe the use of astable multivibrator circuits as an economic and easy-to-do alternative to software control of SMP – and to the best of our knowledge – for the first time. Each circuit provided a square wave supply voltage for triggering either one SMP or for activating the SMP trigger circuits, defining by this the operation the time interval for each SMP. The analytical performance of the proposed assembly was studied with two FIA-like flow systems and successfully applied to the determination of nitrite in natural water samples.

## 2. Experimental

### 2.1 *Reagents*

Milli-Q quality water was used for the preparation of solutions throughout. A 100 mL stock solution of indigo-carmine (Fluka) of 500 mg $L^{-1}$ was prepared as a model sample for preliminarily studies of the repeatability and reliability of a two-channel flow system and diluted appropriately for calibration standards using water as a carrier.

The system was further adapted to the determination of nitrite in tap water applying the Griess reaction. The reagent was prepared from stock solutions of N-(1-naphthyl)-ethylenediamine (NED) 1 g $L^{-1}$ in water and sulphanilamide (SAA) 10 g $L^{-1}$ in 1.2 mol $L^{-1}$ hydrochloric acid. For adjustment of the reagent acidity, hydrochloric acid, 6 mol $L^{-1}$ was used. A stock solution of sodium nitrite of 100 mmol $L^{-1}$ was prepared and standards were prepared daily by dilution. All reagents were stored cooled and in darkness.

### 2.2 *Manifold and spectrophotometric detection*

Both used flow system are given in Figure 1 with tubing dimensions indicated. All parts in liquid contact were of chemical resistant polymers, namely PMMA (connectors), PTFE (tubing), and PEEK (pump manifold). All tubes were of an inner diameter (id) of 0.8 mm.

Two solenoid micropumps of nominal 8 µL and one of nominal 25 µL (types P/N090SP-12-8 and P/N120SP-12-25) purchased from BIO-CHEM FLUIDICS (Boonton, NJ, USA) were used, following denoted Pumps 1, 2 and 3. Calibration by

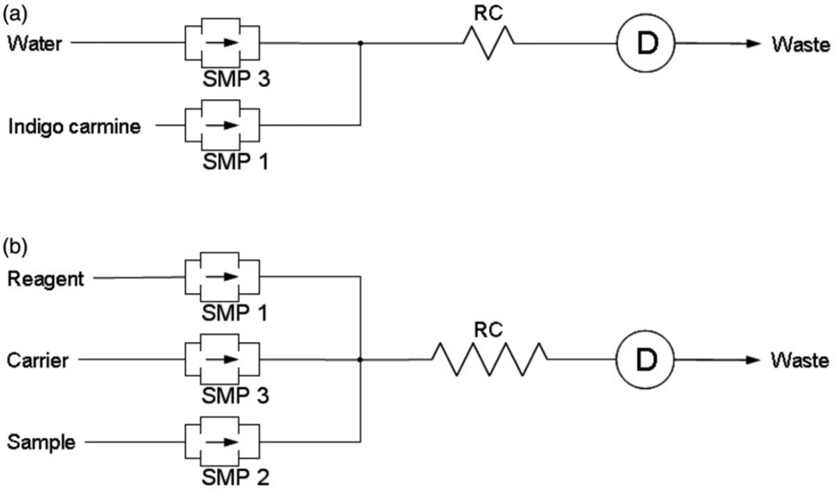

Figure 1. Applied flow systems for preliminary study with indigo carmine (a) and for the determination of nitrite (b). RC: reaction coil (A: 30 cm, 0.8 mm id, B: 200 cm, 0.8 mm id), SMP: solenoid micropump (1 : 8.5 µL, 2 : 27 µL, 3 : 42 µL), D: Detection flow cell (1 cm, 3 id).

weighting the dispense volume of 100 pulses gave pulse volumes of 8.5 µL (Pump 1), 27 µL (Pump 2), and 42 µL (Pump 3), respectively.

For testing the repeatability and sample frequency on a simple flow system, pumps 1 and 3 were connected via a connected by a three-way confluent and further connected via a reaction coil of 30 cm to the detection flow cell. For proving the suitability for an analytical application, all three solenoid pumps were connected via a four-way confluent and a reaction coil of 190 cm was used.

For both systems, an USB-2000 CCD (charge-coupled device)spectrophotometer was used and directly attached to a cuvette support CUV-UV (both Ocean Optics Inc., Dunedin, FL, USA) holding a 1.0 cm path-length flow cuvette from Starna (type 75.3 Q, Essex, UK) connected to the respective reaction coil. A home-made light source based on the combination of a blue light-emitting diode (LED) and a halogen bulb as former described [10] was used and powered by the same source as the SMP (see below).

Dual-wavelength detection was done for compensation of the schlieren effect using the difference of the absorbance values at the analytical and the reference wavelengths as analytical signal throughout. For indigo carmine measurements, an integration time of 4 ms and averaging over 50 single data measurements was done applying a data acquisition frequency of 4 Hz. 610 nm and 750 nm were applied as analytical wavelength and reference wavelength, respectively. For nitrite determination, a data acquisition frequency of 2 Hz was used. 540 nm and 750 nm were applied as analytical wavelength and reference wavelength, respectively. Data acquisition and evaluation was done by the software AutoAnalysis 5.0 (Sciware S.L.).

### 2.3 *Control circuits and methods*

Triggering and powering of the SMP was done by use of circuits based on the chip NE555. The NE555 was used as an astable multivibrator causing a square wave output between

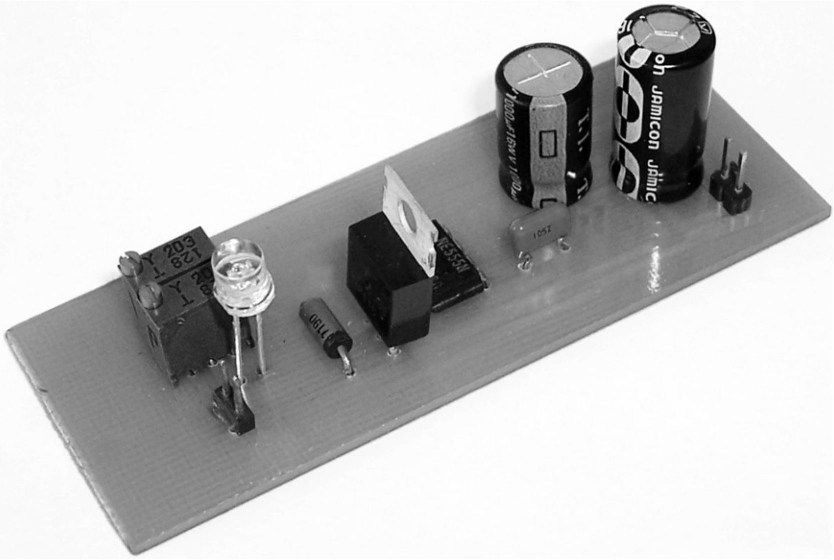

Figure 2. Photograph of an exemplary soldered astable multivibrator circuit.

the states supply voltage (active) and ground (inactive). A photo of one soldered circuit is shown in Figure 2. The scheme of the entire control device and electrical connection of SMP is shown in Figure 3.

Activation and deactivation times were adjustable independently by changing the values of two 10-turn trimmer potentiometers $R_1$ and $R_2$, respectively. For the used fixed capacitor $C_1$, the times can be calculated as follows:

$$\text{Activation time [s]} = \text{In}(2) \cdot R_1 \cdot C_1 \quad \text{Deactivation time [s]} = \text{In}(2) \cdot R_2 \cdot C_1$$

The independency of both characteristic times is enabled by the LED connected in parallel to $R_2$. The diode 1N4001 was required to discharge the counter-voltage induced by the solenoid of the SMP or relay at power release. The output load of the NE555 was reduced the complementation of the trigger circuits B, C, and D with the power transistor TIP41C.

One astable multivibrator circuit (A) was used to trigger the voltage supply time intervals of three further circuits (B, C, D) via a relay. Circuit B was supplied with the relay in position INACTIVE while circuits C and D were supplied with the relay in position ACTIVE. The later circuits were used to trigger the pulsation of the solenoid micropumps itself, driving the carrier, reagent, and sample, respectively. The operation scheme of the three-channel flow system used for the determination of nitrite is given in Figure 4. For electrical supply, a PC power source adjusted to 13 V was used as well as for light source powering.

## 3. Results and discussion

### 3.1 *Preliminary remarks*

The values of the resistances $R_1$ in the circuits used for the control of the pulsation of each the individual pump were adjusted to values about 6.1 kΩ corresponding to activation

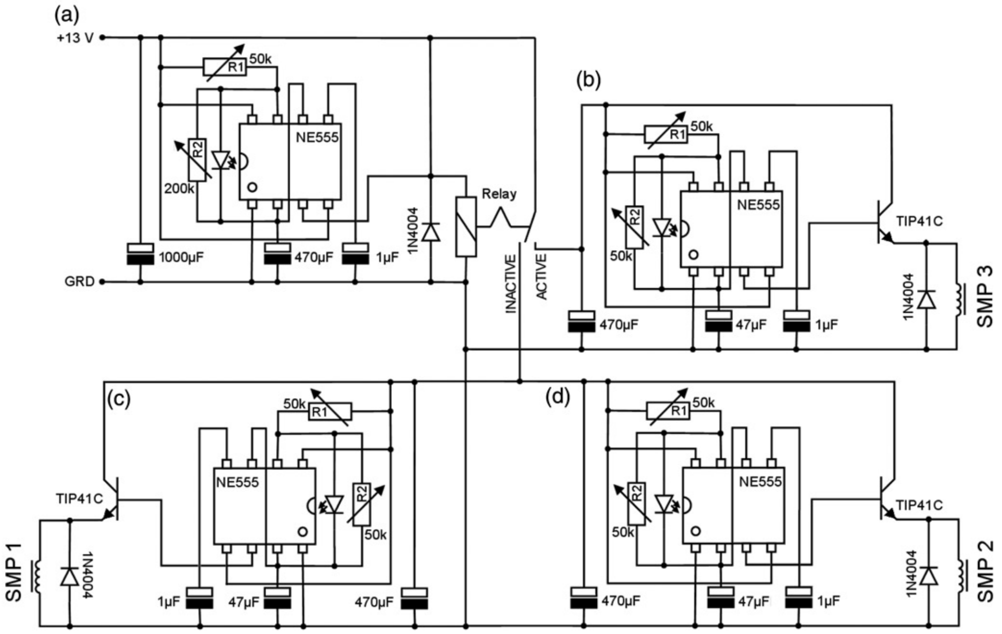

Figure 3. Assembly of four astabilemultivibrator circuits. Circuit A was used to define the time intervals for the supply of the circuits B, C, and D. These were used the powering and triggering of three solenoid micropumps for carrier (SMP 1), chromogenic reagent, and sample (SMP 2 and 3), respectively. The power transistor TIP41C was replaced by a relay in circuit A with positions ACTIVE (circuit B activated) and INACTIVE (circuits C and D activated).

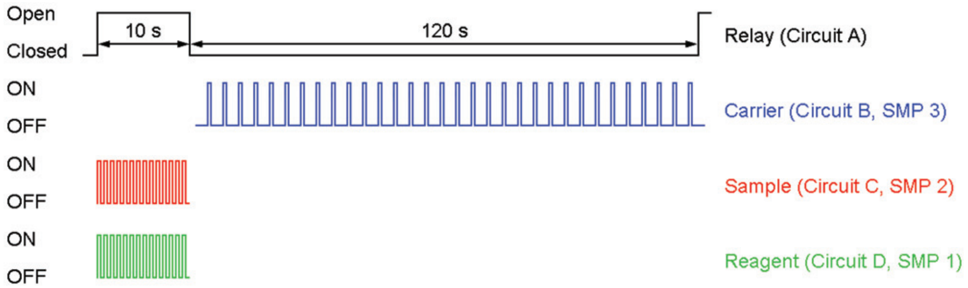

Figure 4. Operation scheme for the determination of nitrite.

times about 200 ms. By this, reliable activation and aspiration was guarantied but simultaneously avoiding excessive heating of the solenoid as a consequence of prolonged operation [11].

## 3.2 *Study with indigo carmine*

Indigo carmine was demonstrated to be a good model substance for spectrometric detection in flow systems. This is because it does not undergo colour change between pH 5

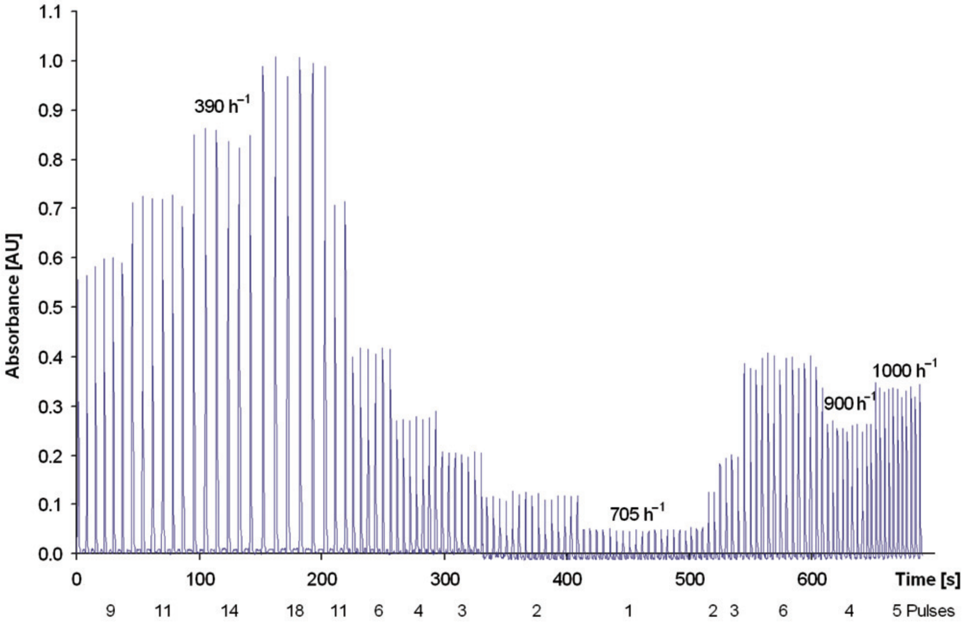

Figure 5. Injection of indigo carmine solution 50 mg L$^{-1}$ (multiple of pulses from SMP 2, each 9 μL) increasing the carrier flow. Absorbance data is given in Table 1.

and 9 and water can be used as carrier without further modifications required. Indigo carmine is easily soluble in water, so adsorption at the inner tubing walls is negligible and finally shows a very high molar absorptivity.

The repeatability of the assembly was tested on a two-channel flow system as shown in Figure 1a by the injection of a multiple pulse volume from Pump 1 (circuit B) of 50 mg L$^{-1}$ indigo carmine followed by the activation of Pump 3 (circuit D) to drive the injected indicator solution towards the detector.

High repeatability was obtained for all tested injection volumes corresponding to a range of 1 to 18 pulses of Pump 1 for injection (about 9 to 81 μL). Peaks and statistical description are given in Figure 5 and Table 1, respectively: The mean relative standard deviation (RSD) was 2.6% of peak height and high injection frequencies of up to 1000 h$^{-1}$ were possible by minimisation of both the activation time of each pump as well as their individual pulsation frequencies.

Owing to the dead volume of the sample-driving solenoid pump and conducting tube, there was a time delay at the sample change of about two injections, which affected the peak height of two peaks. Analysis of discrete samples required, therefore, at least three injections while for continuous operation, i.e. process monitoring, this alteration would be negligible.

### 3.3 *Determination of nitrite in water*

In the following, the flow system was modified by the integration of solenoid pump 2 as shown in Figure 1b. Pumps 1 and 2 were driven by separated trigger circuits (C and D),

Table 1. Data corresponding to Figure 5, mean absorbance and standard deviation in dependency of the number of pulses applied for injection of indigo carmine 50 mg $L^{-1}$.

| No. of pulses | Mean* [AU] | SD [AU] | RSD (%) | Realised sample frequency [1/h] |
|---|---|---|---|---|
| 1 | 0.058 | 0.003 | 4.5 | 705 |
| 2 | 0.123 | 0.008 | 6.2 | 686 |
| 3 | 0.204 | 0.005 | 2.2 | 684 |
| 4 | 0.275 | 0.007 | 2.7 | 679 |
| 4 | 0.269 | 0.007 | 2.7 | 900 |
| 5 | 0.341 | 0.009 | 2.7 | 1000 |
| 6 | 0.410 | 0.008 | 1.9 | 596 |
| 6 | 0.398 | 0.012 | 3.1 | 732 |
| 9 | 0.580 | 0.018 | 3.2 | 500 |
| 11 | 0.717 | 0.008 | 1.2 | 439 |
| 11 | 0.708 | 0.004 | 0.6 | 434 |
| 14 | 0.845 | 0.015 | 1.7 | 390 |
| 18 | 0.989 | 0.014 | 1.4 | 356 |

*Satisfies the regression function: $\text{Absorbance} = 2.21 \cdot (1 - e^{(-\text{No. of pulses}/29.4)})$, $r^2 = 0.999$.

both connected to the relay position INACTIVE. Pump 3 was used for driving of the carrier (water) and the belonging trigger circuit (B) was connected to the relay position ACTIVE. Pump 1 was used for propulsion of the chromogenic Griess reagent while Pump 2 was used for sample.

Consequently, when circuit A output on the supply voltage and thus the relay state are active, both reagent and sample are injected into the flow system while at circuit A output on ground and thus the relay state in inactive, the carrier pushes the mixed solution segment towards the detector.

In order to simplify the system, the trigger circuits of Pumps 1 and 2 were adjusted to the same operation frequency of 1.4 Hz of both pumps. By this, the mixing ratio between reagent and sample during injection was about 1 : 3 (8.5 μL : 27 μL).

For injection, an activation time interval of 10 s was chosen, corresponding to injection volumes for sample and reagent of about 380 μL (10 s × 1.4 Hz × 27 μL) and 120 μL (10 s × 1.4 Hz × 8.5 μL), respectively. Larger volumes – achievable by increasing the activation time of both circuits – did not lead to valuable improvements of peak height but broader peaks owing to lower dispersion.

Since the volume of carrier required for flushing of the flow-manifold depended on activation time of circuit B and triggering frequency, the best strategy was to evaluate the required volume or pulse number (here about 1.2 mL) and to evaluate the response of the reaction on the reaction time. In a former multipumping flow system, the Griess reaction was found to be fast [12], so an injection frequency of about 30 $h^{-1}$ seemed feasible. Consequently, atime interval of 120 s activation of the corresponding trigger circuit B was chosen. During this time, the composite slug of sample and reagent was driven through the reaction coil and detection flow cell. A low operation frequency of only 0.27 Hz was applied for Pump 3 (carrier) corresponding to a reaction time of about 90 s. Longer reaction times did not lead to an analytical response, which would have justified the decrease of the injection frequency.

The composition of the reagent was improved by univariant study of hydrochloric acid, SAA, and NED using a nitrite standard of 40 μmol $L^{-1}$ keeping the physical

Table 2. Data corresponding to the study of the chemical parameters of the reagent used for the determination of nitrite corresponding to hydrochloric acid, NED, and SAA. Mean ± standard deviation of peak heights for a 40 μmol $L^{-1}$ nitrite standard are indicated.

| Acidity [mol $L^{-1}$]* | Mean ± SD | NED [g $L^{-1}$]** | Mean ± SD | SAA [g $L^{-1}$]*** | Mean ± SD |
|---|---|---|---|---|---|
| 1.08 | 0.330 ± 0.014 | 0.02 | 0.154 ± 0.004 | 0.5 | 0.187 ± 0.003 |
| 0.99 | 0.345 ± 0.023 | 0.04 | 0.214 ± 0.004 | 1.0 | 0.234 ± 0.003 |
| 0.90 | 0.344 ± 0.014 | 0.08 | 0.276 ± 0.003 | 2.0 | 0.276 ± 0.004 |
| 0.81 | 0.335 ± 0.015 | 0.12 | 0.304 ± 0.006 | 3.0 | 0.293 ± 0.003 |
| 0.72 | 0.323 ± 0.006 | 0.16 | 0.294 ± 0.004 | 4.0 | 0.298 ± 0.003 |
| 0.63 | 0.330 ± 0.009 | 0.20 | 0.295 ± 0.003 | 5.0 | 0.307 ± 0.005 |
| 0.54 | 0.327 ± 0.008 | 0.30 | 0.272 ± 0.004 | 6.0 | 0.305 ± 0.003 |
| 0.48 | 0.342 ± 0.010 | 0.50 | 0.238 ± 0.004 | 7.0 | 0.311 ± 0.004 |

*Study made with 4 g $L^{-1}$ SAA and 0.1 g $L^{-1}$ NED in the reagent.
**Study made with 0.9 mol $L^{-1}$ hydrochloric acid and 4 g $L^{-1}$ SAA in the reagent.
***Study made with 0.9 mol $L^{-1}$ hydrochloric acid and 0.15 NED in the reagent.

parameters flow rates and time intervals constant. The results are given in Table 2. An increase in the hydrochloric acid concentration of the reagent led only to a slight increase of the peak height of about 2% over the studied range of 0.48–1.08 mol $L^{-1}$ and a concentration of 0.9 mol $L^{-1}$ was used further on. In contrast a clear maximum was found for the concentration of NED studied in the range of 0.02–0.3 g $L^{-1}$ and the optimal concentration of 0.15 g $L^{-1}$ was used further. Finally, for SAA, an increasing peak height was found with increasing concentration of SAA reaching a plateau at about 4 g $L^{-1}$, which was consequently chosen for all further experiments. A high repeatability with RSD of generally less than 2% was found throughout.

The figures of merit were calculated from standard calibration with peaks shown exemplarily in Figure 6. A linear calibration curve with a sensitivity of 5.45 mAU per μmol $L^{-1}$ nitrite was obtained with a blank value below 3 mAU ($r^2 = 0.999$). The limit of detection (LOD) and limit of quantitation (LOQ) were calculated as the concentration yielding the triple and tenfold standard deviation of the standard blank to be 1.3 μmol $L^{-1}$ and 4.3 μmol $L^{-1}$, respectively. A further improvement of the analytical performance could be achieved by the use of a flow cell with smaller flow channel diameter for less dispersion, reaction coil heating for higher progress of the chromogenic reaction, or a higher mixing ratio of sample and reagent.

The system was tested on four well-water samples. The natural nitrite concentrations of samples 1, 2, and 3 were below the LOD, while a concentration of 119 μmol $L^{-1}$ was measured for sample 4. The samples were spiked with 50, 50, 25, and 25 μmol $L^{-1}$ of nitrite. The analytical results are given in Table 3. The recovery values of the spiked concentrations were between 96.3% and 106.2% with an average of 100% ± 4.3%, demonstrating the applicability of the system for real water sample analysis.

### 3.4 *Key benefits and disadvantages of the proposed computer-less SMP control*

Owing to the former denoted dead volume leading to distortion of the first two peaks after sample change, the system is better suited for continuous operation than for discrete samples, i.e. for monitoring applications.

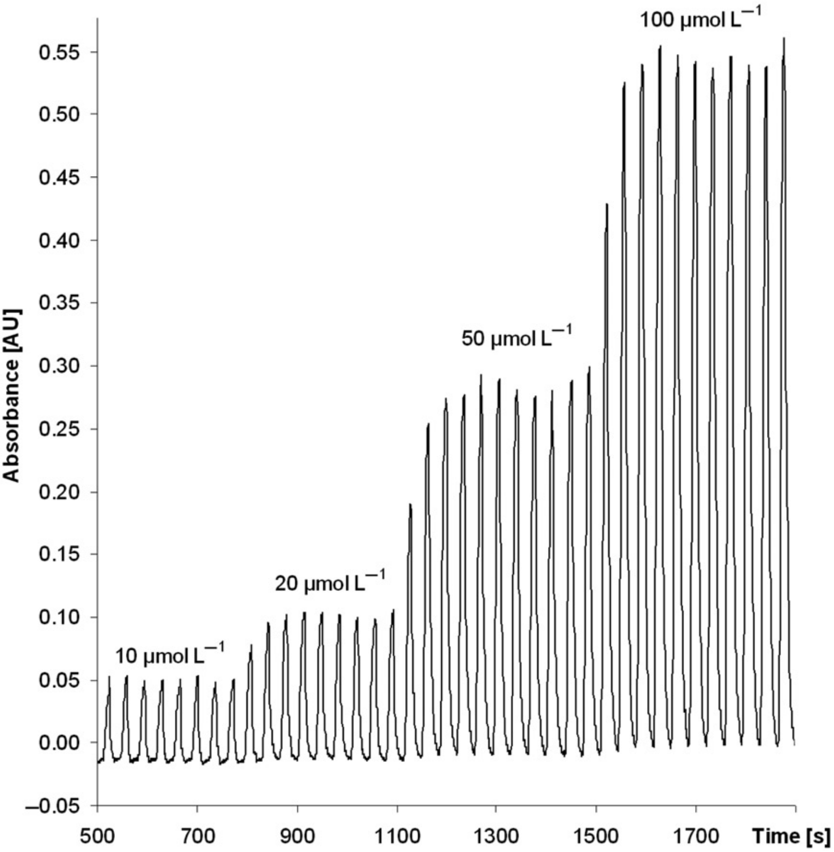

Figure 6. Data from the calibration of nitrite using Griess reagent. Injection volumes 14 µL pulses of both reagent and sample following by 32 pulses of water (carrier).

Table 3. Results from real sample analysis.

| Well water | Spiked concentration [µmol L$^{-1}$] | Found concentration [µmol L$^{-1}$], $n=7$ | Recovery [%] |
|---|---|---|---|
| Sample 1 | 50 | 49.4 ± 1.4 | 98.8 |
| Sample 2 | 50 | 53.1 ± 1.6 | 106.2 |
| Sample 3 | 25 | 24.1 ± 1.1 | 96.3 |
| Sample 4 | 25 | 24.7 ± 0.6 | 98.8 |

Here, an important advantage is that the operation without computer control allows the easy construction of simple analytical systems for each available detector or detection system since there is neither the requirement of purchasing additional software nor of compatibility of software products. This feature is a well-known bottleneck in flow technique automation. Indeed, two computers are often used since there are hardly any versatile software products on the market, which can control both flow system components and detection unit. On the other hand, for the few existing ones, the integration of a control interface for every new instrument implies costsfor programming and thus the profound knowledge of a specialist.

Although economic relay cards are available for solenoid pump control, there are expenses for control software purchase or development and further, there is a commonly observed problem of imprecise timing and interruptions of triggering of these devices whenever another software application is running on the same computer and consequent irregularities in the flow protocol.

The multivibrator circuits further enable further miniaturisation and simplification of remote monitoring and sensor systems, while software control generally requires microprocessors including difficulties of programming, costs, and additional space. On the other hand, the read-out of a detector alone can generally be accomplished by a commercial available data-logger.

For a three-channel flow system, the required solenoid pumps present a highly economic alternative to a peristaltic or syringe pump. The expenses for one multivibrator circuit did not exceed €5, omitting expenses for control software and the required experience of the user. This feature makes the proposed assembly highly attractive for educationpurposes as well as for sample provision from sampling points on industrial or environmental processes. The system show further the important advantage over peristaltic pumps, i.e. that there is no practically no abrasion or permanent squeezing damage of pumping tubes.

There is surely a potential of the presented automation mode for more complex flow systems and further flow operation modes. However, the simplicity of the proposed operation mode of solenoid micropumps is probably the highest benefit to be named. Among future analytical applications requiring only a simple flow system, potentiometric detection systems should be pointed out. Since electrodes are prone to bio-fouling over time, computer-less injection of sample into a carrier or cleaning solution flow can considerably prolong signal stability in monitoring.

## 4. Conclusions

A new automation approach based on solenoid micropumps and suited for standard analytical procedures was presented and successfully applied to the determination of nitrite in different natural water samples. The flow system was based on trigger circuits, which controlledthe flow rates and activation time intervals for each incorporated solenoid pump. Flow and signal reproducibility as well as the found recovery values all met the requirements for analytical applications. The approach demonstrates the potential of using simple electronics for the computer-less control for simple analytical applications especially for at-side process monitoring and for educational institutions.

## Acknowledgements

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