

A pinch-valve interface for automated sampling and monitoring of dynamic processes by gas chromatography-mass spectrometry†

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Hsu Ting,^a Jie-Bi Hu,^a Kai-Ta Hsieh^a and Pawel L. Urban^{*ab}

Gas chromatography (GC) is frequently used in qualitative and quantitative analysis of volatile organic molecules. In its most common embodiment, samples are prepared in the laboratory, and injected into the capillary column using an autosampler. Analytes are separated, and detected by one of several available detection systems, including mass spectrometry (MS). However, this mode of operation is not convenient when monitoring dynamic chemical systems – for example, reaction mixtures. Here we present a facile approach for automated sampling, and subsequent introduction of samples to a GC apparatus in order to enable monitoring of dynamic chemical processes in real time. The proposed system incorporates two pinch valves, a peristaltic pump, a thermoshaker, and an electronic control unit (Raspberry Pi microcomputer) with several relays. The device facilitates dosing small volumes of liquid samples to the injection port of a commercial GC-MS apparatus. A program in C language controls the operation of the pump (on/off, direction of flow) and actuation of the pinch valves, and it initiates GC-MS runs. The system has been characterised with artificial samples, and successfully used in the monitoring of transesterification reactions catalysed by single microbeads containing immobilised lipase, as well as in the monitoring of the extraction of natural products from plant tissue samples. We believe that – following further improvements – this automated approach may be adapted for monitoring industrial processes and environmental analysis.

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1. Introduction

Assaying enzymatic activity has been an indispensable part of studies in biochemistry and biotechnology.^{1–3} Being biocatalysts, enzymes speed up chemical reactions by lowering the activation energy.^{4,5} Enzymes provide improved efficiencies by increasing the reaction rates. Due to their specificity, they ensure high purity of the synthesised products. Appropriate assays need to be developed in order to characterise the catalytic properties of enzymes. Ideally, such assays should enable reaction monitoring within certain periods of time, so that any loss of catalytic activity can be easily noted. Due to the high price of enzymes, it is also desirable that these methods consume as little sample (biocatalyst) as possible.⁶

Gas chromatography (GC)-based methods have been in common use since the 1950s.^{7–9} In order to ascertain satisfactory sensitivity and selectivity, high-performance detectors are normally connected to outlets of GC columns. For instance, mass spectrometry (MS) is one of the popular detectors

hyphenated with GC systems because it can assist identification of the eluting molecules based on their mass-to-charge ratios (m/z) and fragmentation patterns. Owing to their versatility and ruggedness, the hyphenated GC-MS systems are used in routine analyses in environmental science, forensic science, geological sciences, food chemistry, cosmetics and biochemistry – to name just a few application areas.^{10–16}

In general, to obtain high-quality results, and to minimise manual effort, automated sampling devices are combined with GC instruments. Autosamplers make the entire analytical procedure straightforward, and they facilitate high-throughput operation. Clearly, superior repeatabilities can be obtained with robotised autosamplers¹⁷ as compared with the manual injection. GC instruments (sometimes equipped with autosamplers) are often used in discrete analyses – one sample at a time. This mode of operation can provide data on the yield of chemical or biochemical reactions at a given time point.^{18,19} To obtain data revealing progress of a chemical reaction, aliquots of the reaction mixture are withdrawn from the reaction vessel, and transferred to the GC apparatus.²⁰ Such a “low-tech” sampling step conspicuously curtails the advantage of utilizing automated systems since the key part of the analytical procedure (*i.e.* sampling routine) is performed by humans. Moreover, the involvement of manual operation makes it difficult to time the sampling, thus reducing the usefulness of the resulting data in

^aDepartment of Applied Chemistry, National Chiao Tung University, Hsinchu 300, Taiwan. Fax: +886-3-5723764. E-mail: plurban@nctu.edu.tw

^bInstitute of Molecular Science, National Chiao Tung University, Hsinchu 300, Taiwan

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post-run kinetic evaluations. Hence, there is a need to develop automated sampling tools which could easily be coupled with inlets of GC instruments, and enable assaying chemical processes in real time. Such systems could provide optimum conditions (*e.g.* temperature control, stirring) for the studies of dynamic processes, and reduce the delay time between sampling and analysis.

Microcomputers and microcontrollers are vital for the construction of analytical instrumentation.^{21,22} In fact, most commercial instruments incorporate customised microcontroller circuits. In the past few years, many universal microcontrollers have become available. One prominent example is the Raspberry Pi – a single printed circuit board (PCB) microcomputer, which was introduced to the market in 2012.^{23,24} The original purpose of the Raspberry Pi was to enhance education in computer science, electronics, automation, and robotics.²⁵ A plethora of practical uses of this universal platform have emerged soon after the sales had started.²⁶ Typical applications include: data logging, sensing, controlling displays, and automation of household appliances.²⁷ The introduction of the Raspberry Pi – as well as other platforms such as Arduino,²⁸ Netduino,²⁹ mbed,³⁰ or Beaglebone³¹ – has inspired home-grown innovators, and fostered creativity of students. These versatile and easy-to-use electronic circuits nowadays find applications in scientific instrumentation, including the construction of analytical systems.^{32–34}

In this study, we aimed to develop a facile automated injection system for GC to accommodate monitoring of dynamic samples, which would incorporate a minimum number of mechanical parts, and be controlled by the Raspberry Pi microcomputer. The system comprises a control panel, peristaltic pump, two pinch valves, and GC-MS apparatus (Fig. 1). We further demonstrate the applicability of this system in the monitoring of transesterification reaction catalysed by single microbeads containing immobilised lipase enzyme as well as extraction of natural products from small amounts of plant tissues.

2. Experimental section

2.1. Samples and chemicals

A widely available thermostable recombinant lipase (from *Candida antarctica*, expressed in *Aspergillus niger*; E.C. 3.1.1.3) adsorbed on macroporous acrylic resin (specific activity: ≥ 5000 U g⁻¹, microbeads) was used as the main model sample (*cf.* Section 3.3). The samples of lemon and kumquat (*cf.* Section 3.4) were obtained from local grocery shops. Small fragments (~2 mm, <5 mg) of the fruit peel were obtained using a stainless steel blade. Isopropenyl acetate, LC-MS grade acetonitrile, α -limonene, β -pinene, γ -terpinene, and thymol were all purchased from Sigma-Aldrich (St Louis, MO, USA). 1-Butanol was purchased from Merck (Darmstadt, Germany).

2.2. Interface

The interface comprises a Y-junction (P-779, PEEK NanoTight Union, OD 1/16 inch; IDEX Health & Science, Oak Harbor, WA, USA) and two solenoid pinch valves (12 V DC, P/N 075P2NO12-

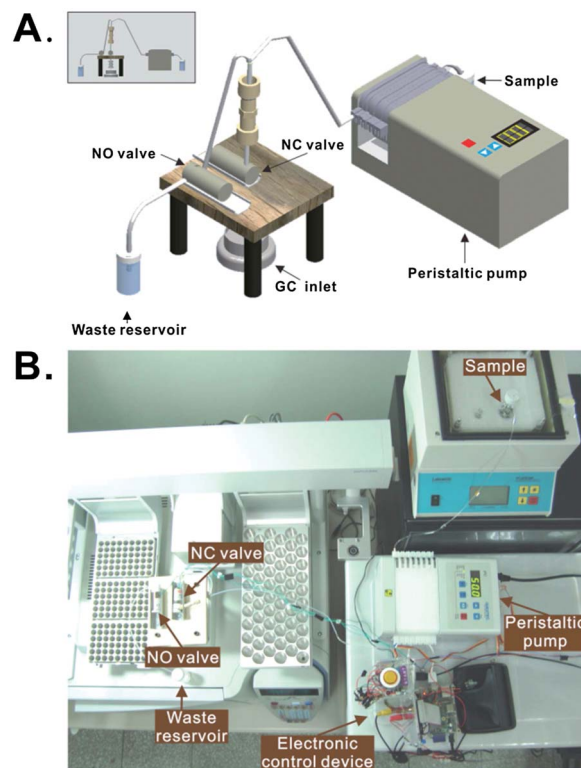


Fig. 1 System for online sampling and sample introduction to GC-MS using pinch valves: (A) device layout; (B) view of the assembled device. NO – normally open valve. NC – normally closed valve. Note: the commercial autosampler (left-hand side) was not used in this study. The lid of the mini-thermoshaker (upper right-hand side) was closed during the experiment.

01S and P/N 075P2NC12-01S; Bio-Chem Fluidics, Boonton, NJ, USA); out of which one is “normally closed” (NC) while the other one is “normally open” (NO; Fig. 1). The valves were attached onto a flat support made of 1 cm thick plywood with a plastic holder and a small amount of modelling clay (Sugru; Form-Form, London, UK), supported by four stainless steel breadboard-type posts (TR75/M-P5, \varnothing 1.2 cm, 7.5 cm long). The sample was delivered by a peristaltic pump (Ismatec ISM936D, IPC Series, 8 channels; IDEX Health & Science). The pump pushed the liquid along a 35.4 cm long section of Tygon tubing (ID 0.25 mm, OD 2.07 mm, cat. no. SC002; IDEX Health & Science), connected to a 28.6 cm long section of polytetrafluoroethylene (PTFE) tubing (OD 1.58 mm, ID 0.3 mm, cat. no. 58702; Supelco, Bellefonte, PA, USA), and – further downstream – to the Y-junction (*via* a 4.8 cm long section of polyimide-coated fused silica capillary; ID 150 μ m, OD 375 μ m, cat. no. 1010-32132; GL Sciences, Tokyo, Japan). The other port of the Y-junction was connected (*via* another 4.8 cm long section of polyimide-coated fused silica capillary, 15 cm section of PTFE tubing, and a 31.4 cm long section of silicone tubing) to the NO pinch valve. The outlet end of the silicone tubing – passing the pinch valve – was dipped into the waste collector (20 mL glass vial). The outlet port of the Y-junction was connected *via* a 6 cm long section of silicone tubing to a 1/16 inch union (IDEX Health & Science). The other side of this union was fitted with a

metal needle removed from a discarded solid-phase micro-extraction (SPME) fibre assembly (23 ga, cat. no. 57284-U; Supelco). The NC pinch valve was mounted on the 6 cm silicone tubing joining the Y-junction and the 1/16 inch union. While on stand-by, the liquid – delivered by the pump – was directed to the waste reservoir because the NC pinch valve prevented its entry to the stainless steel needle. However, when the NC valve got open, and the NO valve got closed, the liquid was directed towards the metal needle, and further on to the injection port of the GC instrument.

The sample vial was placed inside a small thermoshaker (Vortemp 56 EVC; National Labnet Company, Woodridge, NJ, USA) to ensure optimum conditions for the studied process (*e.g.* reaction, extraction). To ascertain seamless operation of the system it is recommended that the Tygon tubing is replaced every day, and the system is rinsed before operation with the solvent used in the analysis for ≥ 15 min. Note that the room temperature during the experiments described here was ~ 20 °C; it is expected that huge variations of ambient temperature could potentially affect the reproducibility of the described method.

2.3. Electronic control system

In order to control operations of the on-line GC-MS injection system, we implemented a single board microcomputer – Raspberry Pi (Type B) with a 700 MHz central processing unit (ARM1176JZF-S) and 512 MB random-access memory (Fig. 2). It was set up with a Linux-related operating system (Debian/Raspbian Wheezy, 2012) loaded from an 8 GB secure digital (SD) memory card (Transcend, Taiwan). The microcomputer was connected to a multifunction input/output extension PCB (Pi PLUS; <http://web4robot.com/>, Parkville, MD, USA) *via* its general-purpose input/output (GPIO) interface to facilitate sending out digital control signals, and further development of the system. For convenience of operation and programming, it was also fitted with standard peripherals (keyboard, mouse, WiFi dongle) and a miniature (~ 4.4 inch) liquid crystal display (LCD) monitor (connected to the RCA-type port of the Raspberry

Pi PCB). The control program – written in C – was referenced to the Pi PLUS function codes and header files provided by the manufacturer (<http://web4robot.com/>), compiled with the GNU compiler on the Raspberry Pi, and deployed. The “sleep()” command was used to time the injection. The Pi PLUS extension PCB was connected *via* digital interface pins to three relay boards (2 relays each; Songle Relay, Ninbo, China). The relays controlled the main functions of the system – including operation of the peristaltic pump (on/off, change of direction), operation of the pinch valves, and sending a trigger signal to the GC-MS instrument. All the PCBs and the monitor were fitted into an acrylic stand (Muji, Tokyo, Japan). The device also featured a “START” button which activated the functions of the program. The flow rate was regulated by adjusting the electric potential on pin No. 5 of the peristaltic pump interface – this was enabled by using a 50 k Ω potentiometer (R_{POT}) in a voltage divider circuit (Fig. 2). For convenience, and to avoid electrical interference, the main components were powered from separate power supplies – Raspberry Pi: 5 V, 1 A; Pi PLUS: 9 V, 1.3 A; pinch valves: 12 V, 1.5 A; monitor: 9 V, 1 A.

2.4. GC-MS apparatus and method

The testing as well as the subsequent analyses were performed using a commercial gas chromatograph (TRACE GC; Thermo Fisher Scientific, Waltham, MA, USA) coupled with a single quadrupole mass spectrometer (ISQ; Thermo Fisher Scientific). A capillary column with non-polar phase comprising 5% phenyl methyl polysiloxane (TRACE SPB-5; length: 60 m; ID 0.53 mm; film thickness: 1.5 μm) was used. The eluting compounds were ionised by an electron ionisation (EI) source operating at 70 eV. Internal calibration of the mass spectrometer was conducted using perfluorotributylamine (Alachua, FL, USA). Helium gas was used as the mobile phase with a constant flow rate of 1 mL min^{-1} at the overpressure of 10 kPa. The injector was kept at 280 °C. Split injection was used with a split ratio of 50. In the first part of the study (enzymatic reaction), the following separation conditions were used: the column temperature was initially set to 40 °C. Following 3 min separation, the temperature was gradually increased reaching 60 °C after 2 min (ramp: 10 °C min^{-1}), then increased to 100 °C (ramp: 35 °C min^{-1}). In the final stage of the run, the temperature was increased to 150 °C (ramp: 20 °C min^{-1}). It was kept constant for 5 min (until the end of the analysis). The mass spectrometer was set to record ions in the range of 42–250 u e^{-1} . The analyte (butyl acetate) species were monitored at m/z 43 while the internal standard (limonene) species were monitored at m/z 93. In the second part of the study (extraction of plant tissue samples), the following separation conditions were used: the column temperature was initially set to 40 °C, and after 3 min, the temperature was increased to 120 °C (ramp: 30 °C min^{-1}). Subsequently, the temperature was increased up to 180 °C (ramp: 15 °C min^{-1}), and it was kept constant for 7 min (until the end of analysis). In this experiment, the mass spectrometer was set to record ions in the range of 40–250 u e^{-1} . The analyte (limonene, pinene, terpinene) species were monitored at m/z 93 while the internal standard (thymol) species were monitored at m/z 135.

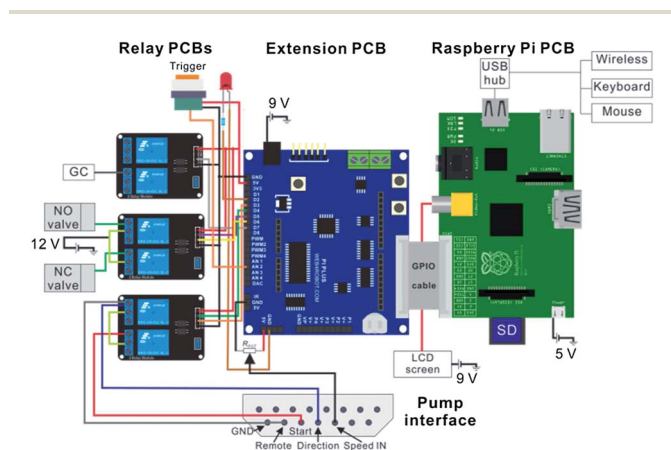


Fig. 2 Simplified layout of the electronic connections of the device microcontroller unit incorporating the Raspberry Pi microcomputer, GPIO extension board, and relay boards.

2.5. Data treatment

The data were acquired using the Xcalibur software (ver. 2.1.0 SP1.1160; Thermo Fisher Scientific), and saved in the “.raw” files. The raw data (extracted ion currents, EICs) were copied from the Xcalibur software (ver. 2.1.0 SP1.1160, Qual Browser) to Excel software (ver. 2010; Microsoft, Redmond, WA, USA), and imported to PeakFit (ver. 4.12; SeaSolve Software, San Jose, CA, USA) for further analysis. The linear baseline was typically fitted according to the 2-point criterion. Fast Fourier transform filter (10–25%) was normally applied. The peaks were fitted using the Gaussian or the Haarhoff – Van der Linde function. The final values were computed using Excel, and then the data were transferred to Origin (ver. 8.0724; OriginLab, Northampton, MA, USA) for final display.

3. Results and discussion

3.1. Construction of the automated pinch-valve sampling system for GC

When designing the automated device for sampling dynamic chemical processes prior to the analysis by GC-MS, we were inspired by the work conducted by Quintana and co-workers³⁵ who developed an ingenious lab-on-valve system for determination of polychlorinated biphenyls incorporating the GC apparatus as a detection tool. In another noteworthy study, Clavijo *et al.* developed a lab-on-valve microextraction system coupled with GC-MS for the determination of polycyclic aromatic hydrocarbons in water.³⁶ Their automated system showed good precision in quantitative analysis (relative standard deviation (RSD) <5%). In the current study, it was of paramount importance to take into account the compatibility of the chemical process to be assayed and the analytical requirements. It was crucial that the studied process could be conducted at preset and constant temperature with shaking/stirring. It was also necessary to make the transfer line as short as possible to reduce the delay time between the sample collection and injection. Finally, it was vital that a defined aliquot of the sample could be introduced to the injection port of the GC apparatus at pre-defined time points. At the same time, unlike in the work by Quintana and co-workers,³⁵ here it was not critical to conduct the sample preconcentration, which would inevitably slow down the analysis of the consecutive sample aliquots obtained from a dynamic system (*e.g.* reaction mixture).

In the final version of the system, we used polymer (Tygon, silicone, PTFE) and silica tubing to aspirate small aliquots of the studied medium and transfer them hydrodynamically to the injector of the GC-MS instrument. The hydrodynamic flow was exerted by a peristaltic pump. The Reynolds numbers along the flow line are in the order of 10–20 (as calculated for 100% acetonitrile), which points to predominance of laminar flow in the sample conduit. Directing the sample to the GC injector was facilitated by two pinch valves (NO and NC; Fig. 1). Efficient control of the flow was achieved by means of the electronic microsystem triggering five relays (see Section 2.3, Fig. 2). All the operations of the system were programmed in a C-language script, and executed according to the optimised time schedule

(Table 1). After setting up, the operation of the system was triggered by pressing a single button (Fig. 2). The samples were then injected, and GC-MS signals were automatically recorded and saved in a sequence for post-run processing. The seamless operation of the system significantly minimised the extent of mechanical and manual tasks to be performed during the analysis.

3.2. Characterisation of the automated pinch-valve sampling system for GC

Fig. 3 and Table 1 present the operation steps of the pinch valves during sample injection. In a nutshell, the 1st step encompasses withdrawal of an aliquot of the liquid sample from the sample chamber. During this step, the transfer tubing is filled with a fresh portion of sample, and any excess sample is diverted to waste. Considering the applied flow rate (1.25 $\mu\text{L s}^{-1}$) and the sampling time (79 s; Table 1), the volume of the sample withdrawn in this step is estimated to be $\sim 99 \mu\text{L}$. In the 2nd step, the sample is directed to the injection port of the GC apparatus for 28 s, which contributes to the withdrawal of a further $\sim 35 \mu\text{L}$ of sample from the sample vial. Eventually, in the 3rd step, the sample is pushed out toward the liner for 6 s, which adds $\sim 8 \mu\text{L}$ to the volume of the sample aspirated by the sampling capillary (see the following paragraph for further discussion of the injection volume). In the 4th step, $\sim 50 \mu\text{L}$ of the “old” sample are returned to the sample chamber, and a small volume of fresh sample (5th step: 15 s, $\sim 19 \mu\text{L}$) is immediately introduced to the flow line. Therefore, $\sim 111 \mu\text{L}$ of the sample solution are consumed in every analysis, most of which is discarded to waste, only a small fraction being dispensed to the GC column inlet. The purpose of withdrawing larger sample volumes than it is actually needed for analysis is to rinse away any residues of the previously analysed sample aliquots from the system. It should be borne in mind that although the 3rd step consumes $\sim 8 \mu\text{L}$ of sample, a lower amount of sample may actually be introduced to the cavity of the GC liner. This is because the distal part of the needle is believed to be filled with pressurised gas after the completion of the 3rd step. In fact, this might help to avoid premature injection of the sample present in the needle due to thermal expansion of the solvent at elevated temperature.

Several experiments were conducted to verify the injection accuracy and precision of the proposed device. In an offline experiment, an analytical balance was used to determine the total mass of the sample (acetonitrile : 1-butanol : isopropenyl acetate = 90 : 9 : 1 (v/v/v)) aliquot pushed out from the needle tip. The obtained value was $7.2 \pm 1.7 \text{ mg}$ (SD, $n = 10$) which corresponds to $9.2 \pm 2.2 \mu\text{L}$ ($d = 781 \text{ kg m}^{-3}$), and remains in good agreement with the value predicted above. The obtained RSD in the injection repeatability test – conducted under ambient conditions (temperature: $\sim 20 \text{ }^\circ\text{C}$; atmospheric pressure: $\sim 101 \text{ kPa}$) – is satisfactory (24%; $n = 10$). However, one shall not exclude a possible influence of the GC operating conditions (temperature inside the liner: $280 \text{ }^\circ\text{C}$; overpressure in the injector: 10 kPa) on the injection. Therefore, we also evaluated the repeatability of the operation of the automated

Table 1 Operation sequence of the custom device for the introduction of liquid samples to the injector of the GC apparatus using pinch valves

Operation	Step (cf. Fig. 3)	Duration/s	Direction of flow	Normally closed (NC) valve (on/off)	Normally open (NO) valve (on/off)
Fill tubing (Tygon and PTFE between the sample chamber and the injection port) with fresh sample	1	79	Normal	Off	On
Fill the injection port with fresh sample	2	28	Normal	On	Off
Injection	3	6	Normal	On	Off
Remove old sample from the injection port	4	40	Reverse	On	Off
Push old sample to the waste reservoir	5	15	Normal	Off	On

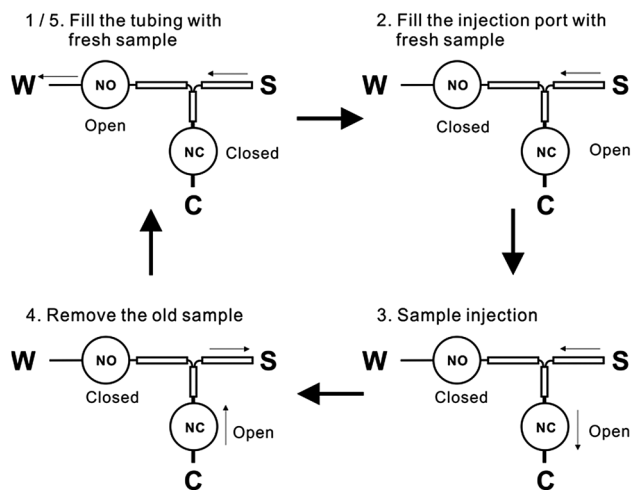


Fig. 3 Schematic diagram of the injection sequence executed using the automated device (cf. Fig. 1 and 2) incorporating two pinch valves (NO – normally open; NC – normally closed). Labels: S – sample, W – waste, and C – column.

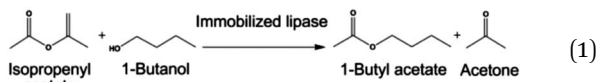
device after coupling it with the GC-MS instrument (Fig. 1). The RSD of the peak areas of a test analyte (1-butyl acetate in the mixture: acetonitrile : 1-butanol : isopropenyl acetate = 90 : 9 : 1 (v/v/v)) recorded during 10 consecutive runs was 27%. When considering the peak areas of the test analyte referenced to an internal standard (α -limonene), the RSD decreased to 13% (Fig. S1[†], EICs for analyte, m/z 43 \pm 0.5 and internal standard, m/z 93 \pm 0.5 u e^{-1}); this was deemed acceptable for further applications. To measure the accuracy of the digital control of sample injection connected to electromechanical actuators (relays, pinch valves), we carried out the following experiment: a video/audio sequence was recorded to capture the action of the relays and the acoustic sounds produced by the relays and the pinch valves. Using video/audio processing software (Ulead Video Studio, ver. 10.0.0110.0 SE DVD; Ulead Systems, Taipei, Taiwan), we estimated the time gaps between the acoustic signals marking the beginning and the end of the injection. The average interval between the “clicks” of the pinch valves was estimated to be 6.0 \pm 0.0 s (n = 3). Considering that the injection time was set to 6 s (in the programme loaded into the Raspberry Pi microcomputer), this result shows that the proposed device provides an accurate and precise control over

the injection time. However, other sources of inaccuracy and imprecision exist; they are believed to be related to the mechanic actuation of the peristaltic pump, tube plasticity, and other causes. In another experiment, we estimated the actual injection volume obtained when the automated injection system was coupled with the GC-MS apparatus (Fig. S2[†]): 1.7 \pm 0.4 μ L (n = 3; based on the comparison of peak areas obtained using the automated system with the peak areas obtained by performing manual injections of different volumes of the standard sample). We think that the lower injection volume in this on-line experiment is due to the influence of gas pressure (10 kPa) inside the liner of the GC-MS instrument. It can be concluded that although the RSDs observed in this study were slightly higher than those obtained with lab-on-valve systems, the injection repeatability can be regarded as satisfactory. In order to bring the RSD values down, and obtain reliable analytical data, it is certainly required to use internal standards (spiked to the samples).

To further characterise the performance of the automated sampling/analysis system (Fig. 1 and 2), we verified its quantitative capabilities. Fig. S3[†] presents calibration plots for 1-butyl acetate, α -limonene, β -pinene, and γ -terpinene. All these compounds are relevant considering the foreseen applications of the system (see Sections 3.3 and 3.4). The coefficients of determination in these plots are within the range: 0.944–0.989 (Table S1[†]); which is believed to be acceptable for the anticipated applications. The increased standard deviation – observed at higher concentration values (Fig. S3[†]) – is believed to be caused by the inaccuracy of curve fitting while measuring areas of highly deformed peaks. The range of the assay was narrowed down to maintain the coefficients of determination at a high level (>0.9; Table S1[†]).

3.3. Application in the monitoring of single-microbead biocatalysis

While many assays for lipases (e.g. spectrophotometry-based)³⁷ focus on the hydrolytic activity of these enzymes,³⁸ for practical applications it is desirable to probe their transesterification activity.^{6,39} Therefore, to exemplify capabilities of the proposed automated system (Fig. 1 and 2) in bioanalysis, first we applied it in the monitoring of a transesterification reaction catalysed by lipase immobilised on macroporous resin microbeads:



It should be noted that transesterification is a crucial step in the production of biodiesel fuel; therefore, it is of considerable relevance when it comes to harnessing green energy.⁴⁰ From the model transesterification reaction depicted in eqn (1), it is evident that transferring the acyl group from isopropenyl acetate onto a molecule of 1-butanol leads to formation of a new ester (1-butyl acetate) and a by-product (acetone). Using the GC method described in Section 2.4, it is possible to separate 1-butyl acetate from all other components of the reaction mixture (Fig. 4).

We further applied the proposed system in the monitoring of transesterification catalysed by varied numbers of lipase microbeads ($n = 1, 2, 3, 4, 5$, and 10). As expected, the enzyme progress curves obtained for different numbers of microbeads have different slopes (Fig. 5). Following 126 min incubation, the relative reaction yield – as expressed by the ratio of the product 1-butyl acetate area vs. the internal standard (*D*-limonene) peak area – recorded for 10 enzyme microbeads – is the highest (Fig. 5, dark blue diamonds). Notably, the relative reaction yield – recorded for 1 enzyme microbead (Fig. 5, red circles) – is only slightly higher than that of the blank (reaction mixture incubated without biocatalyst microbeads; Fig. 5, black triangles). Nonetheless, it is pleasing to note that the amount of product, obtained in the presence of individual microbeads, is detectable. Motivated by this success, we carried out a comparative study of the biocatalytic performance of single enzyme microbeads. As shown in Fig. 6 and S4,[†] the activities of individual microbeads ($n = 8$) are not exactly the same. The reaction

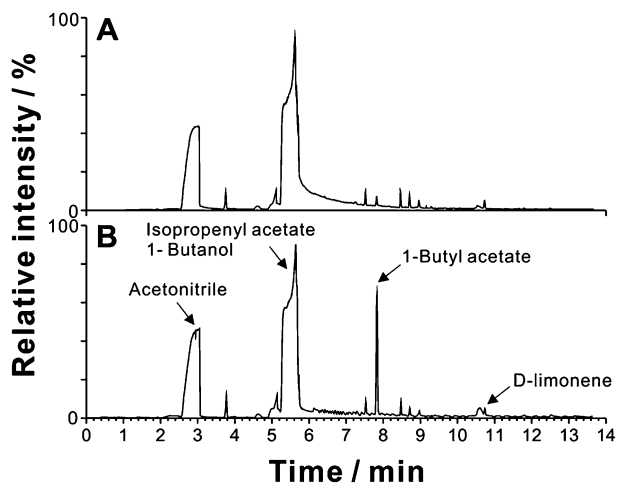


Fig. 4 Full range GC-MS chromatograms (total ion currents, TICs, m/z range: $42\text{--}250 \text{ u e}^{-1}$) of the reaction (cf. eqn (1)) mixture (5 mL; acetonitrile : 1-butanol : isopropenyl acetate = 90 : 9 : 1 (v/v/v)). (A) Blank containing the internal standard (no microbeads added). (B) Sample after 126 min incubation using 10 microbeads of lipase (see Section 2.1). Temperature: 30 °C. Shaking speed: 20 rpm. The peak of the reaction product (1-butyl acetate) can be seen in (B). Internal standard: 10^{-5} M *D*-limonene.

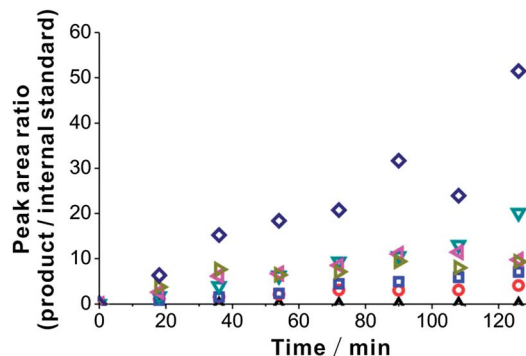


Fig. 5 Synthesis of 1-butyl acetate catalysed by a small number of macroporous resin microbeads with immobilised lipase (from *Candida antarctica*; expressed in *Aspergillus oryzae*) monitored by the setup shown in Fig. 1 and 2. Size range of microbeads: $\sim 400\text{--}600 \mu\text{m}$. Markers: (\blacktriangle , black triangles) 0 microbeads; (\circ , red circles) 1 microbead; (\square , blue squares) 2 microbeads; (\blacktriangleright , green tilted triangles) 3 microbeads; (\blacktriangleleft , green reversed triangles) 4 microbeads; (\blacktriangleright , violet tilted triangles) 5 microbeads; (\blacklozenge , dark blue diamonds) 10 microbeads. Conditions are the same as in Fig. 4. All the data points had been subtracted with the peak area ratio value at time “zero”.

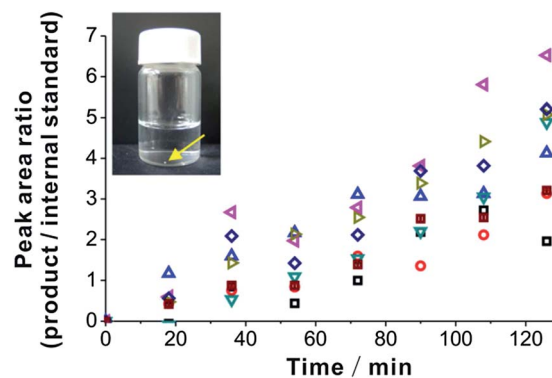


Fig. 6 Single-microbead biocatalysis. Each curve corresponds to one lipase microbead. For EICs (analyte $m/z 43 \pm 0.5 \text{ u e}^{-1}$, internal standard $m/z 93 \pm 0.5 \text{ u e}^{-1}$) of the analyses at 126 min, see Fig. S4.[†] For the calculated reaction rates, see Table S2.[†] Conditions are the same as in Fig. 4. All the data points had been subtracted with the peak area ratio value at time “zero”. The data marked with red circles (\circ , red circles) correspond to the same experiment as the one depicted in Fig. 5. The inset shows the sample vial with a reaction mixture and one lipase microbead (indicated with yellow arrow).

velocities (calculated by fitting linear functions to the datasets in Fig. 6 and feeding the slopes into the calibration equation from Table S1[†]) range from 308 to 432 $\text{nmol L}^{-1} \text{ min}^{-1}$ (Table S2[†]).

Note that the 126 min period corresponds to the initial stage of reaction since the concentration of isopropenyl acetate is in the order of $\sim 0.1 \text{ M}$ and only a small amount of it is depleted (assuming 1 : 1 reaction stoichiometry, eqn (1)). Interestingly, there is no evident correlation between the relative reaction yields (for the 126 min time point) and the specific dimensions of the studied microbeads (diameter, outer surface area, volume; Fig. S5[†]). In fact, one outlier point in Fig. S5[†] indicates the

incidence of particularly large microbeads with disproportionately low enzymatic activity within the microbead population. This unexpected result can be explained by the anticipated heterogeneous immobilisation of lipase molecules on the surface of the acrylic resin. This observation was only possible when conducting single-microbead assay. It can be of use whenever it is required to probe transesterification activities of very small samples of heterogeneous biocatalysts. The heterogeneity of lipase microbeads indicates that multiple microbeads need to be tested one-by-one before a conclusion on the overall quality of the “microbead population” can be drawn.

In conclusion, the proposed method enables observation of the catalytic polydispersity of immobilised enzymes – adding to the conventional bioanalytical toolkit used in biotechnology. Unlike a previously published method,⁶ the current one enables temporal monitoring of transesterifications catalysed by single microbeads, and there is no need that the product absorbs ultraviolet (UV) light. Thus, it can be noticed that the analytical approach presented in this work is different than many conventional assays. The current method targets the transesterification activity of relatively small samples of heterogeneous biocatalysts (microbeads <1 mm). Standard methods often target larger samples, focus on homogeneous catalysis, or probe the hydrolytic activity (as opposed to the transesterification activity of lipase). While GC-MS is an established analytical platform, the current method enables convenient coupling of the GC-MS instrument with a reaction system. Nonetheless, it should also be pointed out that the use of a GC-MS apparatus – as opposed to the use of a UV-Vis spectrophotometer – makes the overall cost of the current method higher. However, the automated system proposed here does not add much to the cost of the existing GC-MS instruments because the sampling device (Fig. 1 and 2) has been constructed using inexpensive parts. It is estimated that the capital cost of the proposed sampling technology is ~10× lower than the cost of a commercial autosampler. Near-infrared spectroscopy is yet another technique for real-time monitoring of transesterifications.^{41,42} It is envisaged that these optical methods – along with the “automated-sampling GC-MS approach” – will form a part of modern analytical toolkit for biocatalyst screening.

3.4. Application in time-resolved extraction of plant tissue samples

To further demonstrate the capabilities of the proposed automated sampling system, we applied it in time-resolved monitoring of the extraction of a real sample (fresh peel of citrus fruit). In this experiment, the sample vial was filled with 5 mL of pure acetonitrile, incubated at 25 °C, and shaken. At time “zero”, a small fragment of freshly obtained fruit peel was inserted to the vial, and the monitoring was started. As the chromatograms were recorded, the increasing signals of certain plant metabolites could readily be observed. For example, the on-line extraction of lemon peel led to the emergence of several signals, including those identified as limonene, pinene and terpinene (Fig. 7 and S6[†]), which are abundant ingredients of citrus fruit.^{43,44} In the case of lemon, the signals of the three

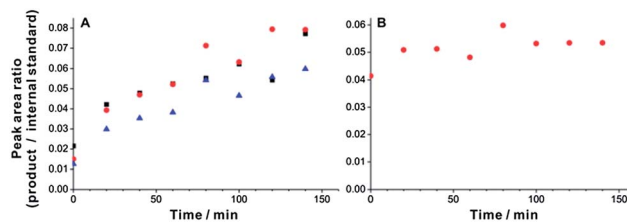


Fig. 7 Monitoring of the extraction of real samples in real time. Time evolution of the extraction profiles. Extraction solvent: 5 mL acetonitrile. Temperature: 25 °C. Shaking speed: 20 rpm. Internal standard: 10^{-5} M thymol. (A) Lemon peel ($m = 3.91$ mg): (●) limonene, (■) pinene, (▲) terpinene. (B) Kumquat peel ($m = 4.00$ mg): (●) limonene. The analyses were conducted in duplicate and representative results are displayed.

identified metabolites became strong after ~30 min extraction, and they were constant until the end of the experiment (Fig. 7A). In the case of the kumquat sample, a substantial amount of limonene was extracted immediately on contact with extractant (Fig. 7B). The time-resolved extraction profiles provide information on the availability of target analytes for extraction. In fact, a previous study – conducted in this laboratory (N.B. also utilising universal electronic modules) – clearly demonstrated the usefulness of time-resolved extraction monitoring to reveal the extractability of sample/specimen constituents.⁴⁵ Although no generalisations can be made based on the limited number of real samples, the current result proves that the method can reveal differences in extractability of selected plant tissue matrices. The extraction monitoring in real time – enabled by the proposed analytical system – can further facilitate optimisation of extraction conditions by reducing the number of manual operations to minimum.

4. Conclusions

We have demonstrated a simple device for automated sampling of dynamic chemical processes, and instantaneous analysis by GC-MS. The device coupled with a GC-MS apparatus is easy to use. It also offers the advantage of reducing the extent of mechanical operations during sampling and analysis. As exemplified in this report, it enables analyses of dynamic processes such as enzymatic reaction and extraction. The optimum conditions for these processes (*e.g.* temperature, shaking) can readily be achieved and maintained. One disadvantage of using this system is that the septum of the injector has to be replaced often in order to prevent leakage of the carrier gas. This problem can readily be rectified by substituting septum with a metal plate to which the needle of the injection system could be soldered permanently. Another consideration is that the current setup is mainly applicable to short term monitoring since a few hundred microlitres are withdrawn from the sample chamber – depleting the medium of reaction or extraction. This issue can be mitigated by using larger volumes of reaction/extraction solvents, in which case the depletion of solvent during sampling would only have a negligible effect on the analysis result. To further improve the quantitative aspects

of the presented method, it is suggested that isotopically labelled internal standards are used as additives to the reaction or extraction mixtures. Nonetheless, when doing so, attention must be paid so as to avoid experimental artefacts (by possible shifting of the reaction/extraction equilibria). Overall, we envisage that the proposed system can further be used for monitoring, optimisation, and mechanistic studies on other chemical and biochemical processes. Following additional alterations to the valve unit assembly and the program, one might also consider coupling this system with other types of analytical instruments (for example, mass spectrometers, without separation columns), which would increase the range of potential applications. It is also interesting to note that – in this study – we have demonstrated the usefulness of a popular microcomputer (Raspberry Pi) in the construction of automated analytical systems, thus expanding the application realm of universal electronic modules in chemistry laboratories. Down this path, we believe that such miniature electronic modules will soon become an indispensable component of the instrumental tackle box in chemistry, enticing industrious analysts into development of automated systems for a wide range of analytical tasks.

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