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Automated on-line liquid–liquid extraction system for temporal mass spectrometric analysis of dynamic samples

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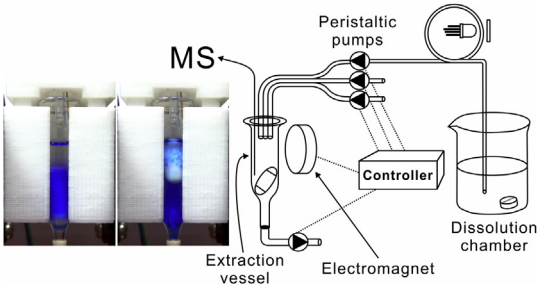
HIGHLIGHTS

- Mass spectrometric analysis normally requires sample preparation.
- Liquid–liquid extraction can isolate analytes from complex matrices.
- The proposed system automates the entire liquid–liquid extraction process.
- The platform enables long-term monitoring of dynamic samples by mass spectrometry.
- It integrates Internet-of-Things features for enhanced ergonomics.

GRAPHICAL ABSTRACT



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ABSTRACT

Most real samples cannot directly be infused to mass spectrometers because they could contaminate delicate parts of ion source and guides, or cause ion suppression. Conventional sample preparation procedures limit temporal resolution of analysis. We have developed an automated liquid–liquid extraction system that enables unsupervised repetitive treatment of dynamic samples and instantaneous analysis by mass spectrometry (MS). It incorporates inexpensive open-source microcontroller boards (Arduino and Netduino) to guide the extraction and analysis process. Duration of every extraction cycle is 17 min. The system enables monitoring of dynamic processes over many hours. The extracts are automatically transferred to the ion source incorporating a Venturi pump. Operation of the device has been characterized (repeatability, RSD = 15%, n = 20; concentration range for ibuprofen, 0.053–2.000 mM; LOD for ibuprofen, ~0.005 mM; including extraction and detection). To exemplify its usefulness in real-world applications, we implemented this device in chemical profiling of pharmaceutical formulation dissolution process. Temporal dissolution profiles of commercial ibuprofen and acetaminophen tablets were recorded during 10 h. The extraction-MS datasets were fitted with exponential functions to characterize the rates of release of the main and auxiliary ingredients (*e.g.* ibuprofen, $k = 0.43 \pm 0.01$ h^{-1}). The electronic control unit of this system interacts with the operator via touch screen, internet, voice, and short text messages sent to the mobile phone, which is helpful when launching long-term (*e.g.* overnight) measurements. Due to these interactive features, the platform brings the concept of the Internet-of-Things (IoT) to the chemistry laboratory environment.

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1. Introduction

Dynamic chemical systems occur widely in nature. In biological specimens, qualitative and quantitative alterations of chemical composition can be observed in the time scales spanning from microseconds to days [1–3]. For example, concentrations of metabolites secreted by microbial cells vary according to the stage of cell growth [4]. In chemical oscillating reactions, the major chemical components alternate between two concentration states [5,6]. Studies of dynamic systems are often carried out to understand fundamental chemical processes, such as reaction mechanisms and kinetics [7,8]. Chemical dynamics can also be observed in the macroscopic world. In fact, quantitative measurements of toxic chemicals present in the environment are crucial for the evaluation of their impact on human health. Monitoring concentrations of chemicals in time is also conducted in the industrial setting to gain insight on the progress of synthetic processes [9]. Moreover, temporal characterization of physical and chemical processes is relevant to applied sciences, such as development and testing of pharmaceutical formulations [10]. Thus, chemical analysis of fast-changing complex matrices in real time has recently attracted considerable attention of the analytical community.

For many years now, mass spectrometry (MS) has been applied in biochemical and pharmaceutical assays. It provides superior sensitivity [11], identification [12], high-throughput [13] as well as quantitative capabilities [14]. Due to these features, MS is particularly suitable for carrying out analyses of dynamic matrices with temporal resolution. However, infusing complex samples to mass spectrometer can lead to contamination of delicate parts of the instrument (ion source, ion guides, focusing elements). Moreover, ionization of some analytes may be suppressed by abundant and readily ionizable matrix components (*e.g.* Ref. [15]). In many cases, in order to obtain high-quality data, and to conduct quantitative analyses, some kind of sample treatment is performed prior to the MS measurements.

There has been enormous progress in the development of micro-scale sample preparation techniques which can be easily coupled to MS. Due to their ability to isolate the compounds of interest from bulk matrices, various extraction techniques have been used to treat complex samples before their infusion to MS systems. For example, mechanic sample disruption/extraction [16] and Soxhlet extraction [15] were coupled to MS via *electrospray ionization* (ESI)-related interfaces. *In-situ* solid–liquid microextraction of analytes can be carried out using the so-called *liquid microjunction surface sampling* (LMJ-SSP) strategy [17,18]. That approach integrates automated sample treatment with rapid and direct analysis under ambient conditions by MS [19]. The invention of the so-called *desorption electrospray ionization* (DESI) encouraged application of MS in the analysis of samples supported on solid surfaces [20,21]. Thunig et al. coupled hollow fiber liquid-phase microextraction and DESI-MS for analysis of drugs present in urine samples [22]. The method called *nanospray desorption electrospray ionization* (nanoDESI) facilitates solid–liquid extraction of surface-supported samples right before the ionization [23], and it found applications in the analysis of organic and biological molecules [23,24]. *Solid-phase microextraction* (SPME) [25] enables trapping analytes onto surface of sampling tools, which can subsequently be subjected to elution with a solvent prior to MS analysis [26,27]. A relatively new method, *slug-flow microextraction* (SFME) enabled rapid analysis of biofluid samples by *nanospray electrospray ionization* (nanoESI) [28]. A *digital microfluidic*-assisted sample processing strategy combined with extraction of analytes was also introduced [29]. It relies on C_{18}-functionalized magnetic beads and enables control of the amount or type of the stationary phase on-the fly. Interestingly, the technique called *extractive electrospray ionization* (EESI) is a mass spectrometric approach used to analyze real samples with minimum or no sample preparation [30]. While it is referred to as "extractive", the ion formation is – in fact – preceded by a different process – fusion of microdroplets [31].

Biochemistry and pharmacology-related samples are normally present in the liquid phase. Thus, they are amenable to *liquid–liquid extraction* (LLE) [32,33]. The conventional liquid–liquid extraction procedures are laborious and time-consuming, which limits analytical throughput of the protocols that utilize this sample treatment technique. Hyphenating LLE with MS can assist analysis of complex samples by reducing interferences and mitigating the risk of instrument contamination. Along these lines, the automated microscale LLE – developed by Raterink et al. – exhibited comparable or even better performance than conventional LLE [34]. (For specialized reviews on extraction procedures, see also refs. [35–40].)

In order to collect multiple MS data points in the time domain, it is necessary to automate the extraction step. In fact, automation is implemented in various areas of chemical analysis [16,41–48]. Automated methods are normally expected to provide not only good reproducibility and repeatability but also high throughput. The Internet-of-Things (IoT) is a network of physical objects incorporating electronics, software, sensors and advanced connectivity which facilitates exchange of information with other connected devices and human [49,50]. The IoT has the potential to improve daily laboratory operations in various ways (*e.g.* automation, safe-guard mechanisms, feedback control), thus reducing the involvement of humans in certain tasks. In this study, we aimed to develop a fully automated liquid–liquid extraction system for unsupervised on-line mass spectrometric analysis of dynamic samples. It incorporates various universal electronic modules (*cf.* [48]) to achieve automation at low cost. The novelty of the proposed platform relates to the fact that – unlike many conventional assays – it enables long-term monitoring of dynamic processes such as drug dissolution. Thus, it may find application in the drug formulation development and testing as well as other areas where it is necessary to follow the dynamics of chemical composition of unstable matrices.

2. Experimental section

2.1. Materials

Ammonium acetate, formic acid, and caffeine were purchased from Fluka (St. Louis, MO, USA). 1-Butanol, methanol (LC-MS grade), and water (chromatography grade), were purchased from Merck (Darmstadt, Germany). Acetaminophen, *N*-acetyl-L-glutamine, ibuprofen, nicotine, phenylephrine hydrochloride, scopolamine, and spermidine were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Microscale liquid–liquid extraction-MS system

The extraction process was carried out in a microscale funnel-shaped vessel custom-fabricated of glass for the purpose of this project (height: 50 mm; inner diameter (center): 6 mm; outer diameter (center): 8 mm; diameter of the collar (upper part): 12 mm; diameter of the outlet section (lower part): <1 mm; glass-blowing workshop, National Tsing Hua University, Hsinchu, Taiwan, Fig. 1). Please note that this design assumes that the extraction solvent is lighter than the sample matrix. Using heavier solvents would require alteration of the design. The extraction vessel was fitted into a holder fabricated from acrylonitrile-butadiene-styrene (ABS) polymer using the 3D printing technology (UP Plus 2; Beijing