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Unattended reaction monitoring using an automated microfluidic sampler and on-line liquid chromatography

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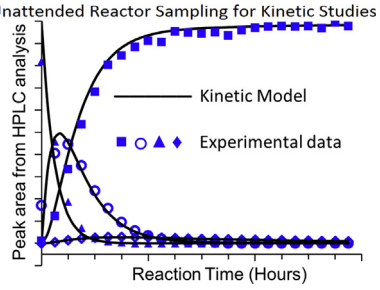
HIGHLIGHTS

- Automated in-process sampling and on-line sample preparation with microfluidic sampler.
- Unattended reaction monitoring with on-line HPLC.
- Microfluidic automated program (MAP) developed to provide extremely powerful yet intuitive control of the instrument.
- Multiplexing capabilities of the instrument shown for Design-of-Experiments (DOE) studies.

GRAPHICAL ABSTRACT



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ABSTRACT

In-process sampling and analysis is an important aspect of monitoring kinetic profiles and impurity formation or rejection, both in development and during commercial manufacturing. In pharmaceutical process development, the technology of choice for a substantial portion of this analysis is high-performance liquid chromatography (HPLC). Traditionally, the sample extraction and preparation for reaction characterization have been performed manually. This can be time consuming, laborious, and impractical for long processes. Depending on the complexity of the sample preparation, there can be variability introduced by different analysts, and in some cases, the integrity of the sample can be compromised during handling. While there are commercial instruments available for on-line monitoring with HPLC, they lack capabilities in many key areas. Some do not provide integration of the sampling and analysis, while others afford limited flexibility in sample preparation. The current offerings provide a limited number of unit operations available for sample processing and no option for workflow customizability. This work describes development of a microfluidic automated program (MAP) which fully automates the sample extraction, manipulation, and on-line LC analysis. The flexible system is controlled using an intuitive Microsoft Excel based user interface. The autonomous system is capable of unattended reaction monitoring that allows flexible unit operations and workflow customization to enable complex operations and on-line sample preparation. The automated system is shown to offer advantages over manual approaches in key areas while providing consistent and reproducible in-process data.

1. Introduction

A significant portion of the lengthy drug development cycle and its high cost [1] is spent in process development and optimization.

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Shrinking development timelines requires greater efficiency in synthetic procedures and unit operations. In many cases, in-line or at-line analytical measurements (also known as process analytical technology, or PAT) increase productivity while providing additional insight and minimizing repetition.

High-performance liquid chromatography, and more recently ultrahigh-performance liquid chromatography (HPLC/LC, uHPLC), are the workhorse tools of pharmaceutical analytical chemistry due to its capability of separating mixtures for qualitative and quantitative process characterization, which is not possible with optical spectroscopy sensors. Further, the timescale of modern ultrafast separations is approaching that of typical sensors [2–4]. Chromatography can provide information on structurally analogous species over a broad range of concentrations, or separate different toxin loaded isoforms in the case of antibody drug conjugates (ADCs).

In-process samples obtained at discrete time intervals are crucial to monitoring and understanding a process. Often, samples need to be diluted, derivatized, or purified prior to the analysis to remove undesired or interfering components and increase the sensitivity of the measurement. A manual approach to these tasks is laborious and becomes intermittent for long reactions leaving voids in kinetic profiles. Consequently, the complete understanding of the process remains elusive. Due to time and personnel limitations, many potential areas of process improvement often remain unexplored. In addition to productivity improvements, eliminating manual sampling may reduce the risk of ingress of oxygen, moisture, or bacterial contamination into the process during sampling while simultaneously affording higher containment of particularly noxious or dangerous chemical species. Scheduled sampling provides consistent and timely collection of data even during unattended (e.g., overnight) operation which can lead to an enhanced understanding of the process.

Researchers have attempted to automate sample extraction and analysis using either in-house developed or commercial instruments to gain the advantages discussed above [5–18]. Some of the earliest published work involved biological processes, which is not surprising since these processes typically utilize aqueous solutions and room temperature operation. This is far simpler in comparison to automated sampling from heterogeneous solutions or saturated solutions at elevated temperatures. Maintaining sterility is not trivial however, when working with biological processes.[1]

Cooley and coworkers showed the value of real-time monitoring to control the operation of purification columns in the manufacturing of synthetic human insulin; only possible *via* on-line LC due to the cycle time and volumes of the fractions [15]. On-line LC for control of commercial-scale purification systems in biological processes is now a fairly standard practice. Researchers have coupled on-line LC with other process sensors to demonstrate the value of multivariate modeling for the characterization and control of these inherently complex processes [9]. Efficiency gains afforded through automation by doing design-of-experiments (DOE) with minimal operator intervention have been demonstrated [10].

Sampling reproducibly from organic synthetic processes can introduce additional challenges due to the variety of matrices and sample conditions. Immiscible phases, suspended solids, the use of possibly incompatible organic solvents, and elevated or sub-ambient temperatures make the extraction and transport of in-process materials more difficult. Despite these challenges, there are many applications of automated sampling from synthetic organic processes in the literature. Sharp and coworkers demonstrated the extraction of synthetic solutions to a mass spectrometer (MS) to characterize development chemistry [18]. Similarly, Dell'Orco and coworkers showed how complete characterization with LC-MS in close to real-time provided mechanistic insights [7,16,17]. Hein and coworkers demonstrated the utility of an on-line LC-MS coupled with Fourier transform infrared spectroscopy (FTIR) for rapid characterization of catalytic reactions [5]. Welch and coworkers used a LC system built for coupling to bench-top experimentation, but it is no longer commercially available [13]. A review described application of on-line MS, with and without LC, for characterizing chemical processes [12]. More recently, the onset of flow chemistry (particularly continuous reactions) may underscore the utility of real-time monitoring for continuous process verification. Maloney [19] and Lambert [11] have described their work with continuous reactors. Wu described the coupling of a micro-sequential injection sample preparation device to a UPLC instrument for amino acid characterization during a mammalian cell culture fermentation [10]. It is apparent that process monitoring with on-line LC can offer significant benefits.

In the last few years, commercial process monitoring devices have appeared, though they are generally focused on sample extraction and dilution (see Supporting Information for a list). While they succeed in improving efficiency, they all have limitations including decoupled LC analysis from sample extraction, restriction to homogeneous solutions, or limited flexibility in the workflow. In most cases, there is a limit in the number and type of operations that can be performed on extracted samples making them inadequate for research and development work which requires flexibility. As reported recently by the Enabling Technologies Consortium, there is a strong need for an on-line reaction monitoring system that combines automated sampling, sample preparation, and on-line LC [20]. The work herein describes development of an automated reaction monitoring system that addresses some of these shortcomings.

The current work is directed at development of a general-purpose tool that can be used in a broad range of sophisticated sample manipulation operations from laboratory to pilot scale equipment. A microfluidic automated program (MAP) with an intuitive user interface has been modified to provide a fully automated sampling and on-line LC system capable of unattended reaction monitoring. A few hardware additions have been made to the microfluidic sampler to add unit operations and to extend its capabilities. The MAP allows complete control of all unit operations with graphical interface and eliminates the need for building sampling sequences at the programming level, a task that is not practical in the context of constantly evolving research and development needs. The MAP interface allows an unlimited number of permutations to the sampling procedure, thereby providing complete flexibility of workflow. The modifications described herein have greatly increased the versatility of on-line LC, providing sufficient flexibility for almost all common fluidic processing demands in chemical development laboratories. When applied to operations in support of large biomolecule process development, the system demonstrated reliable and robust performance. Examples provided show sampling from various scale reactors, including sampling from multiple reactions run in parallel. The mode of operation of the device along with extensive characterization and applications are described. A comparison to manual sampling and analysis is shown. The features and limitations of the current equipment are discussed along with design improvements currently in progress.

[1] https://www.fda.gov/downloads/Drugs/Guidances/ucm070342.pdf Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice, Food and Drug Administration, Rockville, MD, 2004, pp 1–63. https://www.fda.gov/downloads/Drugs/Guidances/ucm070342.pdf.