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On the Optimal Synthesis of Micro Polymerase Chain Reactor Systems for DNA Analysis

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Abstract

The paper makes an overview of the problems related to a new and challenging research direction, namely, the application of the process system engineering approach for the efficient design and operation of micro-bio-chips. It intents to analyse some general aspects of process synthesis including trade-offs, important variables, possible optimisation, energy integration, system's layout and important aspects of systems' design and operation. Presented analysis and discussions are focused on specific type of micro-systems represented by the micro-polymerase chain reactor systems (MPCRS) designed for DNA analysis. It attempts to formulate the problems of primary importance faced by process systems engineers in their attempt to assist the design of highly reliable, flexible and controllable MPCRS.

Keywords: micro systems, DNA analysis, systems engineering, integration

1. Introduction

Nowadays the goal of chemical engineers may very well be equally reformulated as activities of scaling-up from laboratory to industrial scale or scaling-down from laboratory into micro scale, where the processes are much more intensive and efficient. In many cases, the challenges and difficulties associated with the process of scaling down can be even avoided through application of micro-systems. Because of their complexity, dynamic and sensitive environment of operation, the system approach may best suit simultaneous problems consideration. In this case the process systems engineer's activities are focused again at modelling, simulation, optimal design and operation of highly efficient, reliable, flexible and controllable processes. Our strategy is to step on the already developed micro-unit operations and focus on the practical needs of the system as a whole in terms of design sophistication, resources management and minimisation benefiting from the recent advances in process systems engineering and process integration.

2. Micro PCR system

The focus of this study is on a class micro-total chemical analysis systems (μ-TAS) – the micro polymerase chain reaction systems (MPCRS) used for DNA analysis.
The polymerase chain reaction (PCR) is an enzyme-catalysed amplification technique, which allows any nucleic acid sequence to be generated in vitro. Since its introduction

in 1983, PCR has been playing central role with applications in DNA analysis, sequencing of human genome, drug discovery, genotyping for rapid medical diagnostics, food production, validation and control, forensic science and medicine, micro-fuel cells design, environmental analysis, molecular biotechnology, pharmaceutical industry, biological weapons detection, etc. The heart of these systems is the polymerase chain reactor, a bio-chemical catalytic reactor for nucleic acid amplification and replication of certain part of the DNA molecule. PCR is a temperature controlled process conducted by cycling a reaction mix through three-temperature step, typically: *denaturation* at 94^{o}, *annealing* at $50\text{-}60^{o}$ and *elongation* at 72^{o}. A typical PCR reaction thus goes through 20-40 cycles.

What is the motivation to miniaturize the PCR system? (a) Low design cost (small footprints, compact design); (b) Low running cost (reduced reagent consumption); (c) Reduced waste production; (d) High speed analyses (ultimately more rapid thermal cycling), parallel architectures, high throughput; (e) Low contamination risk.

3. Micro-Devices' Specifics

Since transport phenomena are scale-dependent, micro reactors, as a new type of unit operations, have some unique characteristics. Heat transfer coefficients exceed those of conventional heat exchangers by an order of magnitude. This leads to re-evaluation of neglected terms. The heat transfer in the longitudinal direction cannot be neglected because the ratio of wall volume to channel volume is large enough. The increased surface to volume ratio in micro reactors has implications for surface-catalysed reactions. Scaling down theory led to the conclusion that μ-TAS are mainly diffusion-controlled systems (molecular diffusion, heat diffusion or flow characteristics control the process efficiency).

3.1 Scale

Fundamental scaling issue is the so called Cube-Square Law. It simply reflects the fact that volumes scale with the cube of the typical length scale, while areas scale with the square of the length scale. Thus, as a device shrinks, surface phenomena become relatively more important than volumetric phenomena. The most important consequence of this is the observation, that the device mass (and inertia) becomes negligibly small at the micro-scales. Regarding the miniaturization, the arising question is should all components be sub-millimetre scale? Some authors pledge that the perception of shrinking everything down to small scale is misleading for micro reactors. The question is if this is valid for μ-TAS systems?

3.2 Pumping

In μ-TAS reactors fluids flow through a network of micro-channels without turbulence. Main differences in flow conditions depend on pumping strategy that formulates a pressure-driven or shear driven flow with the usual assumption of a slip-velocity condition. The time in this case is regarded as a surface and is proportional to the square of the typical length (tube diameter, or other). The liquid velocity in the case of electroosmotic flow (EOF) is constant across the channel except for the diffuse layer very close to the wall. Unlike EOF, pressure driven flow produces a parabolic velocity profile with high velocities in the channel centre and slow velocities near the wall. The

EOF velocity is independent of the channel cross section dimension; whereas the volumetric flowrate does depend on channel dimensions. A mixture of EOF and pressure driven flows can be practical.

3.3 Mixing

The fluids can only mix by diffusion. Micro-mixers can reduce mixing time to milli- or nano-seconds. For the usual flow velocities in the range of 0.1-1.0 mm/s and Reynolds numbers <10 the mixing of two fluids at a T-junction occurs by inter-diffusion because both fluids retain their laminar flow patterns in the joint flow. If for instance the channel width is of 100 μm and diffusion coefficient of 0.5 m^2/s, the time for mixing of two streams at 1 mm/s will be 100 s, i.e. quite unsatisfactory long time. The more efficient way of mixing is through the "flow-inject-flow", where a slug of one fluid is produced within a flowing stream of a second fluid.

4. Design Implications

The design, i.e. the architecture, is perhaps the most important feature of a microchip as it defines the function and sequence of processes taking place in the device. Miniaturisation of the PCR system provides significantly improved thermal energy transfer than provided by macro-scale systems, thus enabling an increased speed of thermal cycling. The design implications in the micro-scale are quite different compare to the macro scale. For instance, the reserves (over-design, design margins) accounting for possible deviations from the nominal operation condition, can not be used as flexibility compensation in the case of micro reactors, because any over-sizing can substantially affect the function of the device. As stated by Hasebe (2003), the shape factor plays much bigger role in the design of micro devices compare to conventional unit operations. Terms such as perfect mixing, overall heat transfer coefficient, plug-flow, steady state, etc. are usual assumption and their values do not depend on the location in the device. The shape of the device has large degree of freedom and needs to be constrained. New types constraints are to be introduced such as average residence time; residence time distribution and temperature distribution have to be included in the design problem. If shorter residence time in connection device is desirable the system structure is to be amended.

It is difficult to measure and control the flow conditions in the micro device, so an invariant to changes of the physical properties design should be suggested.

5. Modelling and Simulation

The major difficulties in modelling μ-TAS systems are in solving the three-dimensional, time dependent Navier-Stokes equations in complex geometric domain. Thus, efficient flow solvers are critical to the success of the integrated microfluidic device simulators. One example of such integration is the experience in combination of SPACE simulator, realizing time-domain transient analysis and Νεκταr – a general purpose CFD. In many cases the FLUENT code, based on control volume method, was used to solve conservation equations of mass, momentum and energy, but it does not consider the polymerisation changes of DNA amplification process, characteristic for the systems in question. An attempt to develop this missing part was reported recently by Viljoen (2004).

It has to be realized that the anticipated benefit of micro devices development will come from new designs yet unknown; therefore micro devices simulation based on full-physical models may still (but not for long) be more appropriate for exploring new concepts.

6. Process Systems Engineering Impact

PCR system consists of large number unit operations synchronized in particular order. There are filters, mixers, micro pumps, valves, reactor, separators, heaters, coolers, sensors, electrophoresis unit, pipes (channels), etc. Concerns are related to raw materials, products, wastes and utilities management, problems related to detection/measurement, controllability, flexibility, robustness, energy conservation, contamination elimination, etc. The type of micro-unit operation can substantially affect the optimal system structure. The time allowable to transfer material to the next device has to be considered. The objective functions in process optimisation has to be reconsidered (throughput and time are the most important once).
Stepping on the Stokes Research Institute's (http://www.stokes.ie) experience in micro-fluidics hydrodynamics, thermal analysis, measurements in micro-channels, micro-sensors, and the current intensive research in development of main components of micro-bio-chips such as mixers, centrifuges, reactors, detection units and heaters/coolers, our focus is on flexible system design, intelligent control and resources management. Walsh & Davies (2004), conclude that one major problem of the design of a PCR system is the cross contamination. The optimisation criterion targeting increased PCR efficiency is according to Mohamed Gad-el-Hak (1999), the cycle time minimisation, leading further to ramp-rate optimisation. This consequently leads to minimisation of sample fluid volumes (in order to minimise the lumped heat capacity and transition times between temperature zones allowing for controllable residency times in each of the stages of the cycle and user controlled temperatures in each of the zones of the device as suggested by Walsh and Davies (2004)). The new design presented by same authors target handling of large groups of samples and is an alternative to the parallel processing. It tackles many samples in small time intervals.

7. Integration

Much options remains to be explored so that rational tools can be developed for the design and efficient operation of μ-TAS, witnesing an explosive growth during the last decade.

- *Micro-electro-mechano-chemical integration*

Recently micro-electro-mechanical systems (MEMS) have been upgraded into *micro-electro-mechano-chemical systems* challenging a new generation of interactions between with chemical or bio-chemical processes. The impact of such a kind of micro-process-systems-engineering approach would facilitate the identification of interesting micro-systems phenomena, specific properties and design guidelines. The new paradigm for process-systems on chip (PSoC) has analogy with the Systems on chip (SoC) announced by Benini (2003). It brings the problems of micro-network synthesis and micro-network integration and resources management onto the domain of optimal design and optimal operation.

- *Architecture of micro-system (structure, topology)*

As it can be expected, the architecture of micro-system plays substantial role in total efficiency consideration. The production rate can be increased by increasing the number of micro-units operating in parallel. The structure varies from aggregated micro devices, through a combination of conventional and micro devices, to a hybrid system. The production rate can be changed by changing the number of parallel reactor units.

- *"Horizontal" and "vertical" integration*

The development of systems featuring "horizontal" integration by building parallel lanes for high throughput applications, and "vertical" integration by implementing several functions on a single device is the most exciting trend in the microchip world. Often the major push of the integration is towards portability and decreased reliance on external infrastructure.

Benini & Micheli (2003), introduce the *network-on-chip* paradigm for the system on chip design. Their prediction is promoting design methodology to support plug-and-play fashion, reliable operation of interacting components, minimum energy consumption forming micro-network of components and micro-network adapting the protocol stack principle. In other cases the integration concept is related to the number of components in a system. Nguyen (2002) refers to the density of components, concluding that the degree of integration in micro devices follows Moore's law, doubling integration density every 18 months. This growth currently is limited by the photolithography technology, slowing the forecast to doubling integration density every 24 months.

7.1 Control issues

Different from the conventional plants, in the case of micro-systems it is almost impossible to add new measurement devices after construction. Thus, the μ-TAS system and the instrumentation and control systems must be designed simultaneously.

7.2 Heat integration

As reported by Punch et al (2003), the Biot number (the ratio of conductive to convective thermal resistance) is a key parameter in the thermal analysis of micro-PCR device. It was found that the velocities changes within 0.1 – 1.0 mm/s have little effect on the temperature profile along the micro-channels. The design options for controlling the cycle-number *(CN)* and the temperature in three zones of the micro-device are as follows: (a) Solid resistors fixed under the meanders of channels - classical PCR cycler. The obvious sequence of heating/cooling zones assists the efficient energy management. The flow after being heated to 93^{o}C passes without meanders trough 72 degree zone, assisting the beginning of cooling and is further cooled in the meanders of the 58 degree zone. Next, passing quickly through the 72 degree zone the sample is preheated helping to reach required temperature in the following 93 degree zone, and so on. The length of the channel in each zone/the wide of the heater, the volumetric flowrate/pumping abilities and the minimum size of the channel are in strong relation, where the pressure drop and contamination in parallel with the rigid control options are of major concerns. Temperature and *CN* control is possible through the number of heating sections or the meanders; (b) Rotational device of Walsh & Davies (2004) arranges the droplets of sample fluid to float in a carrier immiscible fluid to prevent contamination. A third fluid comes in direct contact with the carrier fluid fulfilling two functions – pumping and

heating/cooling and leaving the channel in particular point. The control is much more flexible and easy. The optimal energy management structure can be found using the Pinch concept. Our experience shows a typical threshold problem case where $\Delta T_{min} < \Delta T_{threshold}$ holds corresponding to non-constrained case. In collaboration with Professor Olaf Strelow, University of Giessen, Germany we developed deterministic methodology and a program code for heat integration of portable two and three-fluid PCR systems (to be reported During the HEFAT conference, Egypt, September 2005). More complex integration problems arise when the system design envisages parallel reactor layers for throughput maximisation and flowshop of DNA analysis, and where the temperature, ramp rate and cycle numbers are different for each layer.

7.3 Synthesis task

An important design optimisation tasks would be: For given process specifications such as number of cycles and temperature ranges, find the best values of design variables and structure of a system that can lead to quickest DNA amplification. Currently the fluidic PCR process is considered as a continuous process, though one should not forget that the time parameter plays a central role. Considering our experience in batch process systems engineering, we found quite similar design tasks that were subject of number of successful approaches commonly formulated as MINLP problems. The immediate design tasks are: (a) Build a mathematical model of MPCRS and perform sensitivity analysis through computer simulation; (b) Design a micro-bio-chip for a wide (but prescribed) range of DNA analysis characterized by high throughput and minimum time.

8. Conclusions

It is clear that combination of expertise in areas such as process systems engineering and micro-bio-chips can lead to interesting amalgamation and confident ability to face the challenge of solving micro-systems engineering problems. Grossman & Westerberg (2000) analysing the future challenges of process systems engineering profession, note that increased attention at the synthesis and design of micro systems may give a rise to design problems that have not received much attention in the past. In line with this prediction we initiated corresponding activities and the results are to be reported soon.

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