

Online Reaction Monitoring System in Microreactor Using Electrospray Ionization Mass Spectrometry: A Methodology for Saving Time and Materials

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Abstract — For online monitoring of chemical reactions in a microreactor, a system implementing the idea of flow injection analysis (FIA) has been realized. Two main functions of the system namely *fast sampling method* and *slow sampling method* have been implemented to control the reaction time via changing the pump flow rate. The purpose is to monitor the formation of a product as well as the decreasing concentration of the reactants according to time. The *slow sampling method* enables multiple sampling. Meanwhile, the *fast sampling method* can reduce working time and reaction materials. The *fast sampling method* has now been improved for further reduction of working resources. An acetylation reaction of N-DL-tryptophan using acetic acid anhydride (AAA) to form N-Acetyltryptophan (NAT) was performed which showed the capability of the method to monitor a change in a chemical reaction over different time periods and to reduce working time and material consumption.

Keywords— online reaction monitoring; microreactor; mass spectrometry; slow sampling method; fast sampling method

I. INTRODUCTION

The combination of microreactors and analytical devices for online monitoring of chemical processes has become popular to industry and research via an increasing number of papers appeared in recent years [1]. This combination exploits the advantages of microreactors such as reaction enhancement, good mixing and control characteristics and the advantages of a suitable analytical device for fast and reliable data acquisition. Therefore, with an online reaction monitoring system, the reaction mechanism and reaction kinetics can be studied conveniently through the monitoring of reactants, products and transient species. At a higher level, reaction conditions can be influenced automatically based on feedback data so that reaction parameters such as reaction rate, product quality or product quantity can be optimized [2].

Different combinations of microreactors and mass spectrometers have been successfully realized. Oosterbroek et al. performed two simple designs, namely a monolithical interface and a modular approach, for direct coupling to a mass spectrometer with electrospray ionization (ESI-MS) [3].

The connection was applied to study the reaction kinetics of β -cyclodextrine and adamantane. Santos et al. directly coupled a microreactor to an ESI quadrupole time-of-flight MS (ESI-QTOF-MS) to study the Ziegler-Natta polymerization of ethane with a homogeneous catalyst [Cp₂ZrCl₂]/MAO (methyl aluminoxane) [4]. Martha et al. combined a coil reactor with atmospheric pressure chemical ionization MS (APCI-MS) for the demonstration of the system capability to simultaneously determine product formation, substrate conversion and catalyst identification [5]. This system implements the flow injection analysis (FIA) concept. Brivio et al. connected an on-chip microfluidic device to a matrix assisted laser desorption ionization TOF-MS (MALDI-TOF-MS) for a real time monitoring of different organic syntheses and biochemical reactions [6]. Clarke et al. coupled a capillary-based reactor to an ESI fourier transform ion cyclotron resonance MS (FTICR-MS) to obtain the kinetic parameters for the hydrolysis of p-nitrophenyl acetate by enzyme chymotrypsin [7]. Barnes et al. implemented an ESI ion trap TOF-MS to study the oxidative degradation of quercetin in an aqueous solution at pH 5.9 and 7.4 [8]. The influence of heat as well as the mechanism and pathway of oxidative degradation of quercetin was investigated. For those systems, the focus is on the analytical functions such as determination of species identification and quantity, reaction rate and reaction mechanism. There is little focus on the automatic operation or on the convenience for the operators. Therefore, a system which automates the operation as well as the data collection and data analysis has been implemented in our laboratory.

A mobile reaction system coupled to an ESI-TOF-MS implementing the FIA idea has been realized [9]. The solution from the microreactor outflow is injected into a carrier stream, which is coupled to the analyzer as shown in Fig. 1. For this FIA system, in order to control the reaction time or reaction stage (or the instance when sample is injected into the carrier stream) the pump flow rate is manipulated. For monitoring a series of reaction stages sequentially in one run (multiple reaction stage), there are two methods namely *slow sampling*

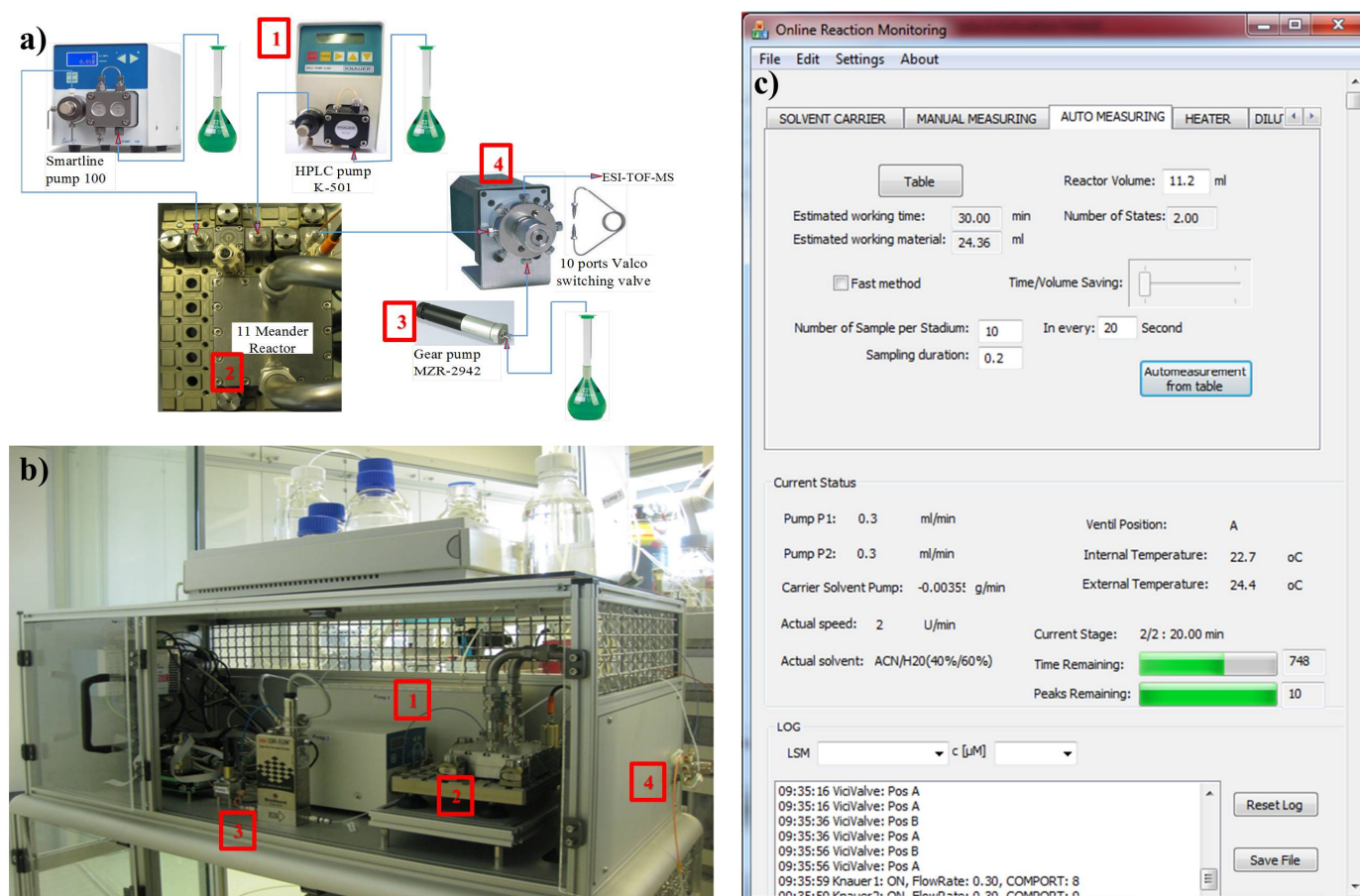


Fig. 1 Online reaction monitoring system. (a) System outline. (b) Real system. (c) Control software

method (SSM) and *fast sampling method (FSM)*. In the *SSM* completely new reactant solutions are used for every reaction stage and the reaction time starts from zero for every new stage. Meanwhile, the idea in the *FSM* is to exploit the reaction time that has elapsed in previous reaction stages to begin for new stage [9]. Therefore, the *FSM* has advantages compared to the *SSM* especially in a lower working time and material consumption. This factor contributes to the economics of the system in long run. However, the current *FSM* has some limitations related to the extension capability for further saving of resources. Therefore, a modified *FSM* has been proposed and tested to solve these limitations. In addition, the total working time and material have also been formulated that can help users to estimate the time and materials required for each running.

II. SYSTEM OVERVIEW

A. System concept

The system includes the following main components (see Fig. 1): The high performance liquid chromatography (HPLC) pumps WellChrom K-501 and Smartline 100 (Knauer, Berlin, Germany) are used for transferring reactants/educts to the 11.2 ml meander reactor (Ehrfeld Mikrotechnik BTS, Wendelsheim, Germany). The solution temperature in this microreactor can be controlled by the F26-ME refrigerated /

heating circulator (Julabo, Seelbach, Germany). After leaving the microreactor the solution enters the 10 ports switching valve assembled with a two position microelectric valve actuator (Valco Instruments Co. Inc, Schenkon, Switzerland). Here, it is sampled and injected to the carrier stream operated by the Micro annular gear pump mZR-2942 (HNP Mikrosystem GmbH, Schwerin, Germany) and the mass flow meter mini CORI-FLOW™ model M13 (Bronkhorst Mättig, Kamen, Germany). The reaction time is considered to stop at this moment. Finally, the sample is transferred to the analytical device (TOF-MS G1969A, Agilent Technologies, Waldbronn, Germany).

B. Data Communication and Control Software

The data communication in the system is performed via RS232 interfaces and from RS232 hubs to the computer by an USB interface. The device software modules *MassHunter Acquisition* and *MassHunter Qualitative Analysis* (Agilent Technologies, Waldbronn, Germany) are used for data acquisition and data analysis from the mass spectrometer.

A software module programmed in C++ is used for the system operation (Fig. 1c). The control software has been modified in order to adapt to different system expansions. The graphical user interface (GUI) has three sections: *control section*, *status section* and *log section*.

The *control section* involves different tabs according to the individual modules and their functionalities. In the Microsoft Foundation Classes (MFC) project, each tab is managed by one class and one dialog. They are combined by a tab control placed on the main dialog. The *status section* monitors the current status of all functional components. The *log section* is used for saving and archiving the detailed activities of the system.

C. Main functions

The system can be operated in *manual mode* and in *automatic mode*. In *manual mode*, users can set flow rates for the educt pumps and inject samples directly using the multiport valve. In *automatic mode*, there are the *SSM* and *FSM* in which flow rates for the educt pumps are calculated and samples are automatically taken based on the reactor volume and reaction stages. In the *SSM*, for every reaction stage, the flow rates are set based on the whole reactor volume and the current reaction stage. The reaction time starts from zero (Fig. 2a). Meanwhile, the *FSM* exploits the reaction time that has elapsed in previous reaction stages to begin for a new stage [9]. In other words, the reaction time of a new reaction stage (except for the first stage) does not start from zero (Fig. 2b). The flow rate (Q) for every two reaction stages (starting from stage 2) is set to be of the smaller flow rate (or flow rate of the later reaction stage with larger time), so that total additional time to perform the two reaction stages equals the later stage only or $\Delta T_{2n} + \Delta T_{2n+1} = T_{2n+1}$. In general, the result of this method is a time reduction of all even stages. However, the algorithm is quite complicated. Also, the formula for calculating the additional reaction time (ΔT) to a new stage has two cases for odd and even reaction stages with the even stage depending on the afterward stage, which may not always be available. This dependence also limits the extension capability of the method via setting the flow rate for every n reaction stage to be equal to the smallest flow rate in each group. Therefore, a new algorithm to overcome the mentioned limitations would be necessary.

III. MODIFIED FAST SAMPLING METHOD

A. A simple case

Consider the reaction volume to have channel-type with equal cross area so that the flow condition is identical over the reactor. A simple case of the modified algorithm for the purpose of understanding is sketched as in Fig. 2c. Not as the previous idea that starts the afterward reaction stage at a randomly position, the new idea begins by dividing the reactor volume (V) into 2 equal parts by 3 nodes (from 0) and starts timing from the middle of the reactor (Node 1). At this point the current reaction stage has elapsed for a certain amount of time. For example, when the first reaction stage set at 60 s has reached, at the middle of the reactor, the reaction has elapsed 30 s. Therefore, to reach the second reaction stage of 120 s, the reaction solution needs to move from the middle point to the sampling point in just 90 s more. In other words, an amount of 30 s is saved for the second reaction stage. It is similar for the third stage that has to wait 150 s more instead

of 240 s because the reaction has been started for 90 s at the middle point. In general, the additional time ΔT_i to stage i is: $\Delta T_i = T_i - \Delta T_{i-1}$ (with $i > 1$), which is also the saving time to stage $i+1$. Similarly, by starting a new reaction stage in the middle of the reaction volume, the required materials for every following stage are also reduced by 50%. This is feasible by reducing the flow rate according to the half-required material and the additional time ΔT_i .

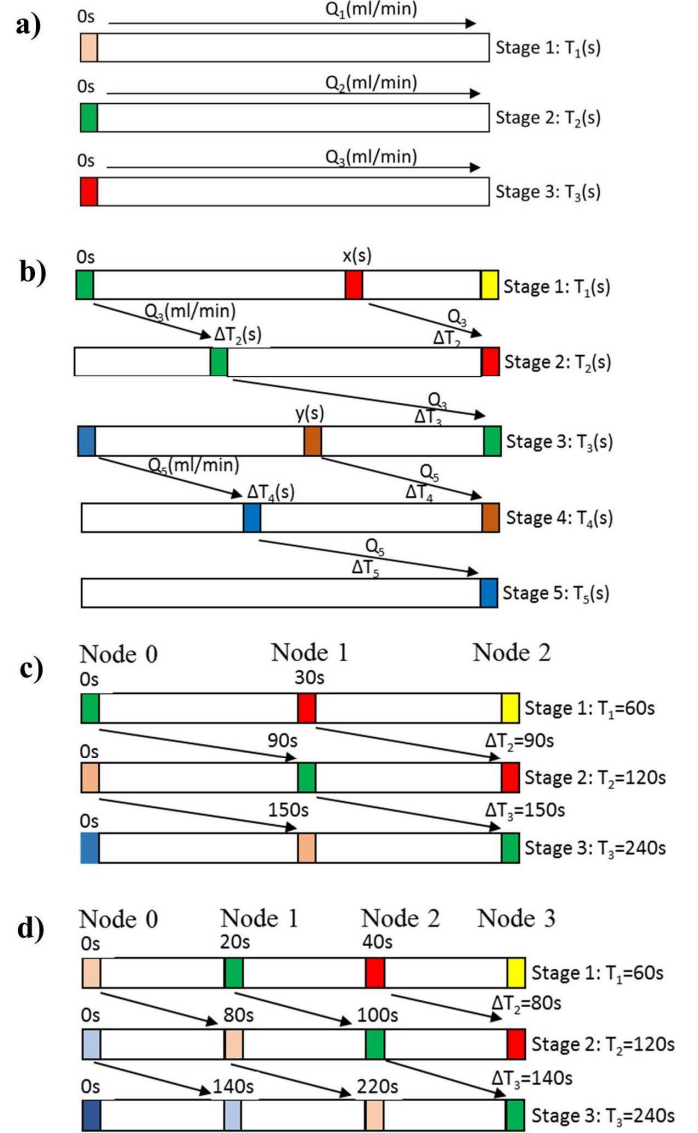


Fig. 2 Sampling method: a) *Slow sampling method*. b) *Old fast sampling method*. c) *Modified fast sampling method (M=2)*. d) *Modified fast sampling method (M=3)*

B. Extension of the algorithm

The philosophy of reducing the waiting time and reaction solution can be extended by further dividing the reactor volume into 3, 4 ... or to any M equal parts by $M+1$ nodes (from 0). For reaction stage n (T_n), the reaction time will be counted starting from the elapsed reaction time at node $M-1$ of stage $n-1$ ($X_{n-1,M-1}$). Therefore, additional time to reach

reaction stage n is $\Delta T_n = T_n - X_{n-1, M-1}$ (with $n > 1$). Similarly, materials required are also reduced to one volume portion (V/M). In that case, the flow rate for the both educt pumps will be changed according to this volume portion (V/M) and the additional time (ΔT_n). Total working time and total reaction materials required for every reaction series of the two methods are formulated as in equation (1), (2), (3) and (4). The algorithm are illustrated in Fig. 2d with $M=3$.

Total working time and material consumption in the *SSM*:

$$T_{slow} = T_1 + T_2 + \dots + T_n = \sum_{i=1}^n T_i \quad (1)$$

$$V_{slow} = V_1 + V_2 + \dots + V_n = n * V \quad (2)$$

Total working time and material consumption in the modified *FSM*:

$$T_{fast} = T_1 + \Delta T_2 + \dots + \Delta T_n = \sum_{i=0}^{\text{floor}\left(\frac{n-1}{M}\right)} T_{n-M*i} + \frac{\text{mod}\left(\frac{n-1}{M}\right)}{M} * T_1 \quad (3)$$

$$V_{fast} = V_1 + \frac{V_2}{M} + \dots + \frac{V_n}{M} = \frac{(M-1)*V}{M} + n * \frac{V}{M} = (M-1+n) * \frac{V}{M} \quad (4)$$

IV. EXPERIMENTAL

The method has been tested through the acetylation of N-DL-tryptophan using acetic acid anhydride (AAA) to form N-Acetyltryptophan (NAT) with the internal standard N-Formyl-DL-tryptophan (NFT). In addition, a comparison among the sampling methods is also presented.

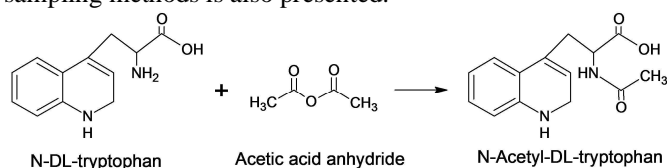


Fig. 3 The acetylation of N-DL-tryptophan to N-Acetyl-DL-tryptophan

Materials and methods: Material details and settings for the MS are as described in [9]. The main highlight includes: Solution 1 includes 20 mg/L N-DL-Tryptophan (MW=204.2) and 2.5 mg/L N-Formyl-DL-tryptophan (MW=232.24, internal standard) in acetic acid. Solution 2 is a 200ml mixture of 20 ml/L acetic acid anhydride (MW=102.09) and acetic acid. Solution 3 is a mixture of acetonitrile and ultrapure water (40/60%) used as carrier solvent.

The MS was operated in negative ion polarity; the scanning rate is 1 spectrum/s and the mass range is from 100 to 3,200 m/z. The drying gas was Nitrogen with a flowrate of 10 L/min. The source temperature was at 350°C and the nebulizer pressure is 40 psig. The following voltages were applied: capillary 3,500 V, fragmentor 175 V and skimmer 65 V.

System settings: The system settings for every experiment are as follows: flow rate ratio of the educt pumps (1:1); reaction volume (12.179 mL); carrier solvent flow rate (0.4 mL/min); reaction stages (1.5, 3, 4.5 minute). The first experiments using a chemical reaction were performed for demonstration of the *FSM* with $M=2, 4$ and 6 equal portions of the reactor and the *SSM*. Each method was repeated 3 times.

By including the heating module, the same reaction was performed at 25°C and 55°C for further comparison of the *FSM* ($M=2, 4, 6$) and *SSM*. Each test was repeated for three times.

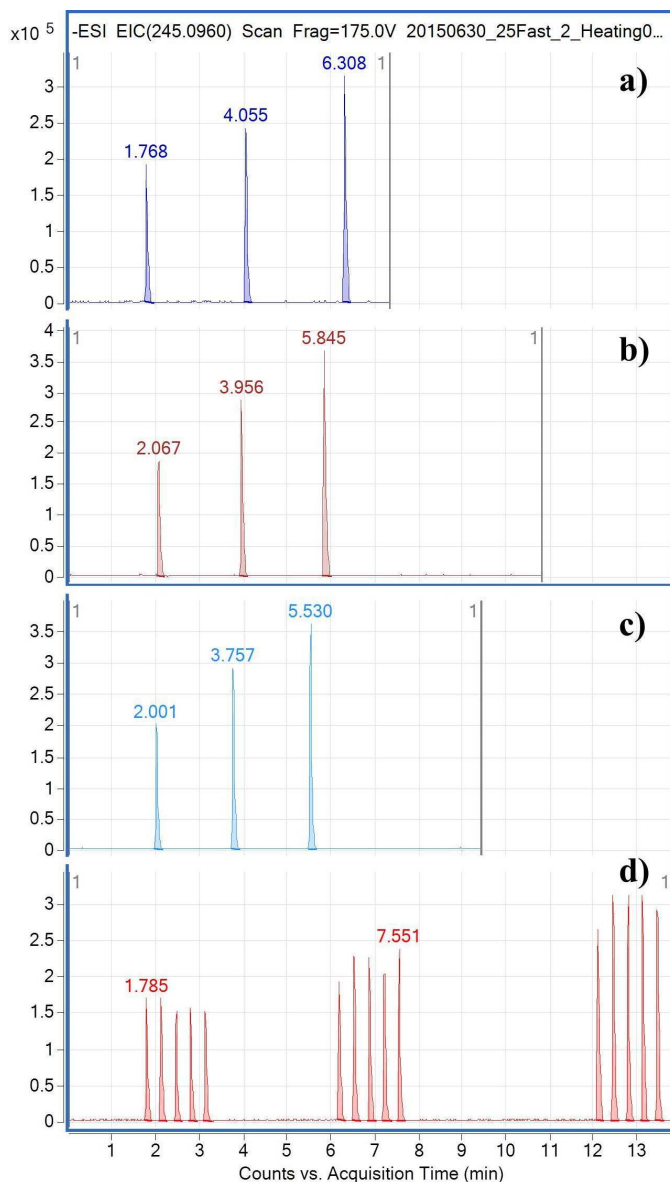


Fig. 4 (a) *FSM* ($M=2$) with product signal. (b) *FSM* ($M=4$) with product signal. (c) *FSM* ($M=6$) with product signal. (d) *SSM* with product signal

V. RESULTS AND DISCUSSION

Measurement results with both sampling methods are shown in Fig. 4. As can be seen, in plot (a), (b) and (c), peaks' density increases as M increases, which is equivalent to more time and materials saved. Those waiting times are visually smaller than those of *SSM* in (d). Moreover, the plot is logical that there is an increase of product, decrease of reactant (*not shown*), and stability of internal standard (*not shown*) when reaction time increases. Further comparison about the efficiency of the modified *FSM* with the old *FSM* and *SSM* are shown in the table. In general, both algorithms for fast

sampling offer better reduction in time and material over the *SSM*. Performance of the old fast algorithm is similar compared to that of the modified *FSM* with $M=2$ (39.6%/39% in time and 45.4%/44.4% in volume respectively). However, the modified algorithm with larger M ($M=4$, $M=6$) is better in performance than the old algorithm (54.5%, 57.9% in time and 66.7%, 74.1% in volume respectively). Moreover, repeat accuracy of the modified *FSM* is better than that of the old *FSM* with relative standard deviation value always smaller than 23% compared to 30% of the old method. Therefore, the new algorithm offers more effective and flexible way in saving resources over the previous *FSM*.

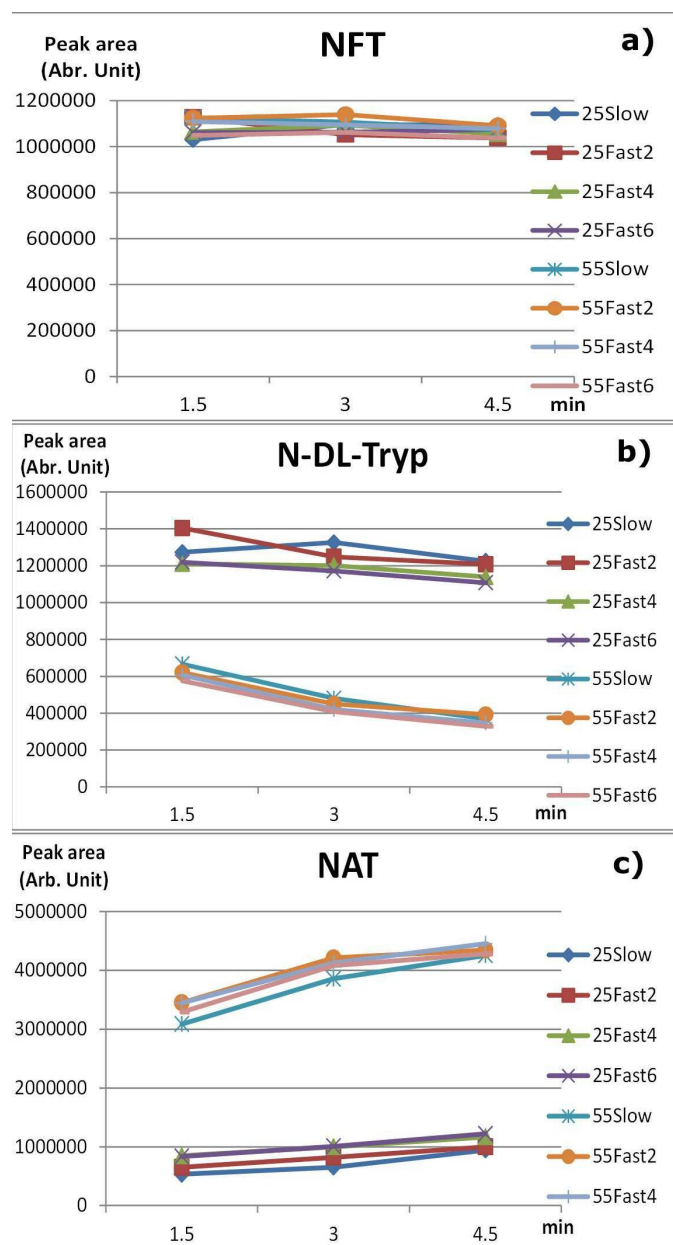


Fig. 5 Measurement results for different reaction temperatures (25°C-55°C) and reaction time (1.5 – 3 – 4.5 min) using the *SSM* and *FSM* ($M=2, 4, 6$): (a) Internal standard. (b) Educts. (c) Product

A further comparison in performance between the modified *FSM* and *SSM* at 25°C and 55°C is also presented in Fig. 5. Each plot represents the results of internal standard (a), educt (b) and product (c) measured by the modified *FSM* (with $M=2, 4, 6$) and *SSM* at 25°C and 55°C. The effects of different reaction temperatures and reaction times on chemical reactions are clearly visible such as the increase of product formation as temperature and time increase; the decrease of educts concentration or the stability of the internal standard with time and temperature.

In the *FSM*, measurements with $M=2$ give similar results to those measured with $M=4$ and $M=6$ with standard deviations at 25°C in range of 1.41%-11.53% and 1.64%-7.57% at 55°C. Those results of the *FSM* are similar to results measured by the *SSM* with standard deviation in range of 1.77%-18.4% and 1.77%-6.7% at 25°C and 55°C respectively. They are indicated in the plots via different groups of lines in which each group represents measurements in similar reaction conditions but different sampling condition.

TABLE I
COMPARISON OF *SSM*, *FSM* (OLD) AND DIFFERENT PARTITIONS OF THE MODIFIED *FSM*.

Sampling method	Slow	Fast (Old)	Fast (Modified)		
Reactor is considered as M equal parts	M=1	M=2 (Not equal)	M=2	M=4	M=6
Max. sample number/reaction stage	No limit	1	1		
Reaction stage (min)	T=1, 2, 3, 6, 10, 20, 30, 60, 90				
Total working time (min)	222	134	135.5	101	93.5
Time reduced over slow sampling method (%)	-	39.6	39	54.5	57.9
Total reaction volume of all stages (ml)	99	54.1	55	33	25.7
Volume reduced over slow sampling method (%)	-	45.4	44.4	66.7	74.1
Repeat accuracy: RSD values (%)	1.6-15	10-30	1.7-3.7	1.1-23.5	0.8-18

Remarks: In theory, it is possible to number M up to any value, however, signal peaks from the sampling may overlap or the flow rate may be too low for the pumps to operate accurately. The accuracy of the algorithm depends on the accuracy of the pumps as well as the external physical effects to the flow. In addition, due to including different flow rates in one reaction stage, the reaction time of solution at every position in the microreactor of the *FSM* is not as stable as in the *SSM* but drifts continuously. Therefore, the method is not suitable for multiple sampling.

VI. CONCLUSION

An online reaction monitoring system for a flexible monitoring of chemical reactions and a modified *FSM* have been presented. Experiments on monitoring the acetylation reaction have confirmed the impression of the modified *FSM* in saving resources over the previous *FSM* and the *SSM*. In general both sampling methods have logically shown their capability to monitor a change in a chemical reaction over different time periods. They can also be applied to other

similar systems that have tube-shaped reaction volume and reaction time is controlled by flow rate. The *SSM* with multiple sampling capabilities is more economical in measurements that require repetitions. Meanwhile, the *FSM* is more suitable for fast data acquisition of a reaction or when there is a limitation in time and materials.

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