KINETIC STUDY OF AN ON-CHIP ISOCYANATE DERIVATIZATION REACTION BY ON-LINE NANO-ESI MS

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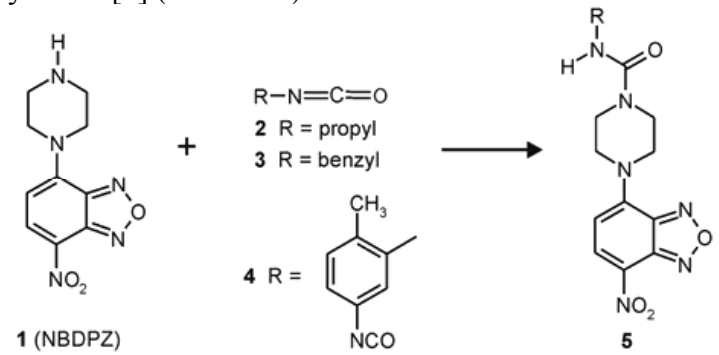
ABSTRACT

A high-throughput method is presented for the study of reaction kinetics by nano-electrospray ionization mass spectrometry (nano-ESI MS). The reaction of propyl isocyanate (**2**), benzyl isocyanate (**3**), and toluene-2,4-diisocyanate (**4**) with 4-nitro-7-piperazino-2,1,3-benzoxadiazole (NBDPZ) (**1**) to yield the corresponding urea derivatives (**5**) was carried out in a continuous flow glass microchip. Real-time monitoring of the reactions was done by nano-ESI MS. Rate constants of 1.6×10^4 M^{-1} min^{-1}, 5.2×10^4 M^{-1} min^{-1}, and 2.5×10^4 M^{-1} min^{-1} were determined for isocyanate **2**, **3** and **4**, respectively.

Keywords: Microreactor, nano-ESI MS, reaction kinetics, real-time monitoring.

1. INTRODUCTION

Microreactors offer a good alternative to lab-scale equipment for the study of reaction kinetics. They allow low reagent consumption and fast mixing [1,2] as well as a continuous flow operative mode and real-time analysis.[3,4] Microfluidic chips have been used to study kinetics of biochemical systems [5,6] on various time scales, mainly using optical detection techniques.[7] Due to the ease of fabrication and their compatibility with aqueous phase biological systems, polymers are the material of choice for the fabrication of chips in most of the studies reported in literature. However, polymer-based microreactors might be less suitable for carrying out organic reactions. In this paper a chip-based nanospray interface [8] is used as a high-throughput platform to study the kinetics of the reaction of NBDPZ with isocyanates [9] (Scheme 1).



Scheme 1. Reaction of NBDPZ (1) with propyl isocyanate (2), benzyl isocyanate (3), and toluene-2,4-diisocyanate (4) to give the corresponding NBDPZ-urea derivatives (5).

2.EXPERIMENTAL

Reactions were carried out in "two inlet" chips (Figure 1a), having various channel lengths (Table 1). "Three inlet" microreactors (Figure 1b) were used to perform control experiments and to study ion suppression phenomena. All microreactors were fabricated by

standard glass processing [8]. NBDPZ (**1**) was synthesized and purified according to Karst *et al*. [9]. Isocyanates were purchased from Sigma Aldrich (Steinheim, Germany). On-chip reactions were studied by directly coupling the microreactor to a Micromass (Manchester, UK) LCT Electrospray time-of-flight mass spectrometer (ESI-TOFMS) and monitoring the decrease of the NBDPZ signal intensity at m/z = 251 in time upon reaction of NBDPZ (**1**) with each isocyanate. Spectra were acquired in the positive ion mode at a capillary voltage ranging between 1.8 KV and 2 KV, at a sample cone of 35 V, and an extraction cone voltage of 2 V. The source temperature was kept constant at 100 °C and the cone gas flow between 80 and 90 L h^{-1}.

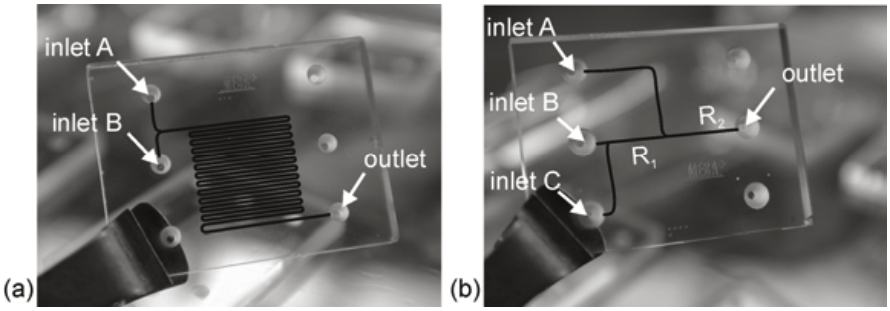


Figure 1. Photographs of the (a) "two inlet" and (b) "three inlet" chips used to study reaction kinetics and ion suppression phenomena, respectively. All microchannels are 50 μm wide and 20 μm deep.

Table 1. Lengths (L) and volumes (V) of the microchannel in the "two inlet" devices and residence times at various flow rates.

Flow rate (nL min^{-1})	L = 7.1 cm	L = 10.5 cm	L = 19.1 cm	L = 28.4 cm
	V = 71 nL	V = 105 nL	V = 191 nL	V = 284 nL
100	0.7 min	1 min	1.9 min	2.8 min
80	0.9 min	1.3 min	2.4 min	3.5 min
60	1.2 min	1.8 min	3.2 min	4.7 min
40	1.8 min	2.6 min	4.8 min	7.1 min
20	3.6 min	5.2 min	9.6 minn	14.2 mi

3. RESULTS AND DISCUSSION

Figure 2 shows the experimental reaction profiles for the on-chip reaction of NBDPZ (**1**) with monoisocyanates **2** and **3** and diisocyanate **4**, determined by ESI-MS by varying the total injection speed of the reagent solutions from 20 to 100 nL min^{-1}. All reactions were carried out injecting on-chip equimolar (5×10^{-6} M) solutions of **1** and the corresponding isocyanate in acetonitrile. The experimental data were fitted to a second order kinetic model (Figure 2) using equation 1. Rate constants (Table 2) were determined by applying a least squares optimization routine.

$$\frac{1}{[A]} - \frac{1}{[A]_0} = kt \qquad (1)$$

[A] is the concentration of NBDPZ (**1**) at a given time, $[A]_0$ is the initial concentration of NBDPZ (1), t [min] is the reaction time, and k is the reaction rate constant. The rate constants determined by chip-based continuous flow experiments are consistent with those determined using batch lab-scale procedures (see Table 2).

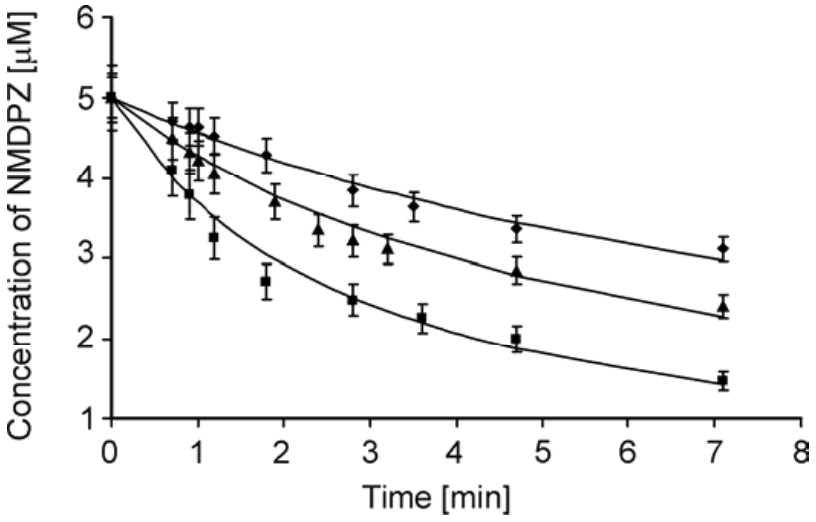


Figure 3. Reaction profile of the on-chip reaction of NBDPZ (1) with (♦) 2, (■) 3 and (▲) 4, and corresponding fits to a second order kinetics model (solid lines).

Table 2. Reaction rates determined by on-chip experiments (k_{chip}) and lab-scale experiments (k_{lab}) and their ratio (k_{chip} / k_{lab}).

Isocyanates	K_{chip} (M^{-1} min^{-1})	K_{lab} (M^{-1} min^{-1})	K_{chip} / K_{lab}
2	1.6×10^{-4}	4.2×10^{-3}	3.8
3	5.2×10^{-4}	1.6×10^{-4}	3.3
4	2.5×10^{-4}	5.6×10^{-3}	4.3

4. CONCLUSIONS

This work demonstrates that continuous flow microfluidic devices provide a valuable tool to carry out and study reactions in a very efficient way, increasing process safety and reducing both reagents consumption and sample handling. The small reaction volume, where reagents mix under laminar flow conditions at very short diffusion length scales, offers a high degree of reaction control contrary to macroscale turbulent systems. Using "three inlet" microreactors allows studying systematically both ion suppression phenomena and the effect of various parameters on sample ionization, which are all required to study reaction kinetics by ESI mass spectrometry in a justified way.

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