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# Continuous process for click reactions using glass micro-reactor functionalized with $\beta$ -cyclodextrin

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#### Introduction

The trend to apply surface-modified materials is increasing rapidly in modern science and technology. Applications of such modified surfaces for example, in catalytic process have the potential of several advantages over traditional chemical methods for example, low amount of reagents needed, less or no waste production along with financial savings.<sup>1</sup> Different materials for example, silicon, glass, quartz, and organic polymers are used for manufacturing such functional surfaces. Among these materials, glass is more attractive because it is optically transparent at wavelengths >320 nm and has a low intrinsic fluorescence. Moreover, it is readily available at low cost, has high mechanical stability, and easy surface modification techniques are known for this material.<sup>2,3</sup>

The click reaction of azides and acetylenes has gained a high popularity since its discovery in 2001.<sup>4</sup> The resulting 1,2,3-triazoles are interesting heterocyclic compounds and have found numerous applications in organic, pharmaceutical, and biological chemistry. Traditionally, click reactions are conducted in batch process but some examples of micro-level flow process have also been reported in last few years.<sup>5</sup> Flow process has many advantages over batch process for example, large surface area-to-volume ratios, small dimensions leading to good mixing, easy heat transfer, easy to scale up etc.

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#### ABSTRACT

A micro-reactor based on glass micro-chip functionalized with  $\beta$ -cyclodextrin was prepared and used to develop a flow process for click reactions of aromatic azides and acetylenes. Products with 97–99% yield were obtained within a few minutes. Modified surface remained stable and almost no degradation was observed after 50 h of continuous use.

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 $\beta$ -Cyclodextrin ( $\beta$ -CD) is a cyclic oligosaccharide composed of seven  $\alpha$ -1.4-linked glucopyranosyl units. In its hydrophobic cavity it can include various molecules or parts of molecules of appropriate size and chemistry by reversible formation of a host-guest complex. While there are many reports on the application of natural<sup>6,7</sup> or modified<sup>8,9</sup>  $\beta$ -CD (powder) as a catalyst for enhancing the efficiency<sup>10,11</sup> in different chemical reactions applications of  $\beta$ -CD as a catalyst is not common in click reactions. Shin et al.<sup>11</sup> and Kaboudin et al.<sup>10</sup> used  $\beta$ -CD and Cu<sub>2</sub>- $\beta$ -CD in click reactions and reaction time was decreased significantly. These results encouraged us to prepare a micro-reactor based on glass micro-chip (GMC) with its micro channels functionalized with β-CD. This modified GMC was used to develop a flow process for copper catalyzed azide-alkyne cycloaddition (CuAAC) click reactions of aromatic azides with aromatic or aliphatic acetylenes. To the best of our knowledge, it is the first report on a flow process for click reactions at room temperature using GMC.

#### **Results and discussion**

#### Functionalization of glass micro-chip with β-CD

GMC surface functionalization was started by formation of terminated epoxide mono-layer (Scheme 1) and characterized by elemental analysis and X-ray photoelectron spectroscopy (XPS). The narrow-scan XPS spectrum (Fig. 1a) of  $C_{1s}$  region from epoxide terminated surface displayed a signal at 285.0 electron volt (eV) corresponding to C–C and C–Si bonds and a signal at 286.15 eV



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Scheme 1. Surface modification of glass micro-chip.

corresponding to C–O bonds.<sup>12</sup> The measured (C-C + C-Si)/(C-O) ratio of 0.49 is in agreement with the theoretical stoichiometric values of 0.50 indicating successful modification of glass surface. Changes in elemental composition (Table 1) also support XPS results. The thickness of the formed epoxide layer, calculated using Eq. 1, was 0.91 nm and is slightly higher than the length of 3-glycidoxypropyl) trimethoxysilane (GOPTMS) molecule (0.88 nm, as calculated with Chem3D), confirming that the formed layer was still a monolayer:

$$I_{\rm si} = I_{\rm si}^{\infty} \, \exp\!\left(\frac{-d}{\lambda_{\rm si,c} \cos\!\theta}\right) \tag{1}$$

Variables,  $I_{si}$  (the absolute silicon peak intensity),  $I_{si}^{\infty}$  (the absolute silicon peak intensity of unmodified glass), d (the thickness of the adsorbed layer),  $\lambda_{si,c}$  (the attenuation length of Si<sub>2p</sub> electrons in the hydrocarbon layer), and  $\theta$  (the electron takeoff angle), were calculated from XPS data as described in.<sup>13</sup>

Subsequently, the epoxide coated GMC was converted into amine terminated surface by reacting with ethylenediamine. In XPS spectrum (Fig. 1b), three signals corresponding to C–C+C–Si (285.0 eV), C–O (285.68 eV), and C–N (286.87 eV) appeared. Excellent agreement between measured value (0.32) for (C-C+C-Si)/(C-O+C-N) and theoretical (0.33) indicates a successful reaction corresponding to 95% conversion of the epoxides (**2**) to amine groups (**3**) on the surface. In addition, appearance of a signal of organic nitrogen at 400 eV from the resultant monolayer (not shown here, see electronic Supplementary data) and elemental composition (Table 1) also indicate that reaction was very successful.

Terminal alkyne groups were attached by reaction of amine terminated surface (**3**) with pentynyl chloride. In  $C_{1s}$  narrow-scan XPS spectrum (Fig. 1c), the signal at 285 eV corresponds to C–C + C–Si linkages while another signal at 286.51 eV corresponds to overlapped signal for C–O and C–N linkages. Theoretical value of (C– C+C–Si)/(C–O+C–N) is 1.38 (calculated according to the structure shown in **4**) and is in good agreement with experimental value of 1.56 corresponding to 89% conversion of amine (**3**) into alkynyl terminated monolayer (**4**). Appearance of additional signals in N<sub>1s</sub> region at 402.70 eV along with usual nitrogen signal at 400 eV (Fig. 1d) indicate that terminal amine was converted into quaternary ammonium cation.<sup>14</sup> Relative ratio of two N<sub>1s</sub> signals was 0.92 (theoretical value 1.0) and shows that 92% of the terminal amine (–NH<sub>2</sub>) were converted into quaternary ammonium cations ( $\equiv$ N<sup>+</sup><sub>1</sub>). All these results confirm successful reaction of pentynyl chloride with amine terminated monolayer.

At the end of this reaction, acetylene terminated surface (**4**) was converted into  $\beta$ -CD (**5**) and phenyl (**6**) terminated surface by click reactions. C<sub>1s</sub> narrow-scan XPS spectrum of  $\beta$ -CD functionalized GMP (**5**, Fig. 1e) showed two signals corresponding to (C–C + C– Si) at 285 eV and (C–O + C–N) at 286.63 eV. Ratio of (C–O + C–N)/ (C–C + C–Si) (theoretical 7.13, experimental 4.53) indicate that 63.55% alkynyl groups were clicked with  $\beta$ -CD-N<sub>3</sub>. Relatively low conversion was probably due to steric hindrance of bigger sized  $\beta$ -CD-N<sub>3</sub>. Moreover, Increase in C content from 43.23% to 52.63% and N from 2.24% to 5.53% along with decrease in Si content from 29.56% to 20.37% after click reaction of alkynyl terminated mono layer (**4**) with  $\beta$ -CD-N<sub>3</sub> (Table 1) also indicates the successful immobilization of  $\beta$ -CD on GMC.

 $C_{1s}$  XPS narrow-scan spectrum of phenyl terminated GMC surface (**6**) also shows two signals (Fig. 1f). Signals at 285 eV and 286.60 eV correspond to C-C+C-Si and C-O+C-N linkages respectively. Theoretical (1.44) and experimental (1.06) values of (C-O+C-N)/(C-C+C-Si) show that 78.26% terminal acetylene

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**Figure 1.** XPS spectra for different steps of glass micro-chip functionalization: narrow-scan  $C_{1s}$  spectrum of epoxide (a, **2**) amine (b, **3**) and alkynyl terminated monolayer (C, **4**),  $N_{1s}$  narrow-scan XPS spectrum of alkynyl terminated layer (d, **4**),  $C_{1s}$  narrow-scan spectrum after immobilization of  $\beta$ -CD on glass micro-chip (e, **5**) and  $C_{1s}$  narrow-scan XPS spectrum of phenyl terminated mono-layer on glass micro-chip (f, **6**).

#### Table 1

Elemental composition and relative number of different bond linkages at different stages of glass micro-chip functionalization with β-cyclodextrin and phenyl terminated monolayer and after 50 h use in a continuous process for click reaction of benzyl azide and phenyl acetylene

Substrate	Elemental comp. (atomic %)			C-C + C-Si bonds		C-O + C-N bonds		
	Si	0	С	Ν	Ther.	Exp.	Ther.	Exp.
Unmodified glass micro-chip (1)	41.19	58.81						
With epoxide terminated layer (2)	31.47	31.41	37.12	0.00	2	1.97	4	4.03
With amine terminated layer (3)	28.61	29.56	39.04	2.79	2	1.93	6	6.07
With acetylene terminated layer (4)	29.56	24.98	43.22	2.24	14	13.36	9	9.64
With $\beta$ -CD terminated layer (5)	20.37	21.47	52.63	5.53	8	11.76	57	53.24
With phenyl terminated layer (6)	17.06	27.20	51.33	4.41	26	22.60	18	21.40
<b>5</b> , After 50 h use	20.46	21.51	52.52	5.51	8	12.94	57	52.06

groups (**4**) were converted into phenyl groups (**6**). Changes in elemental composition (Table 1) also support these results.

# Application of modified GMC as a micro-reactor for click reactions

A continuous process for click reaction of benzyl azide and phenyl acetylene was established using micro-reactor based on GMC (Table 2). Benzyl azide and phenyl acetylene (in *t*-BuOH) were injected into the reactor from one inlet while CuSO<sub>4</sub>·5H<sub>2</sub>O and sodium ascorbate (in water) from the other inlet. Product (**7**) was collected at the receiving end, extracted with ethyl acetate, and analyzed by NMR spectroscopy. Conditions for concentration of reagents and injection rate were optimized using different concentrations (0.1–1.5 M) of benzyl azide and phenyl acetylene (data not shown here) and found that 1 M solution of benzyl azide and phenyl acetylene injected at the rate of 5  $\mu$ L min<sup>-1</sup> with 0.05 M Cu-SO<sub>4</sub>·5H<sub>2</sub>O (5 mol % per acetylene group) and 0.2 M sodium

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Application of unmodified and coated with epoxide, amine, acetylene,  $\beta$ -CD, and phenyl terminated monolayer glass chip as a control in click reaction of benzyl azide and phenyl acetylene



ascorbate (20 mol % per acetylene group) gave product with 98–100% yield.

The role of  $\beta$ -CD as a phase transfer catalyst in click reactions is already known.<sup>10,11</sup> To prove that  $\beta$ -CD cavity forms inclusion complex with aromatic azides or acetylenes that enhances the click reactions, Shin et al.<sup>11</sup> recorded <sup>1</sup>H NMR of  $\beta$ -CD mixture with ben-

zyl azide and phenyl acetylene in water. A downfield variation in β-CD protons was observed. This deviation was more prominent for H-5 proton of β-CD ( $\Delta \delta$  = -0.099 with benzyl azide and -0.107 with phenyl acetylene), which is located inside the cavity at the narrow side of β-CD. These observations indicate that β-CD acts as a phase transfer catalyst and speeds up the reaction by making a host–guest complex with the aromatic ring of azide or acetylene compounds.

To prove that high yield (99%) in click reaction of benzyl azide and phenyl acetylene in few minutes was obtained due to catalytic effect of β-CD immobilized on GMC and not due to any other functional group or due to length of micro-channel itself, un-modified GMC (1) and GMC functionalized with epoxide (2), amine (3), acetylene (4), and phenyl (6, having triazole but not CD) terminal mono-layer was also used for click reaction of benzyl azide and phenyl acetylene. Results (Table 2) show that there was some back ground reaction (22-24%) in case of un-modified GMC or in case of epoxide (2) and amine (3) terminated layer. This back ground reaction is not surprising because all reagents were supplied in soluble form (water/t-BuOH). Little bit higher yield (36% and 31%) in case of GMC functionalized with acetylene (4) and phenyl (5) terminated layer was probably due to Cu ions bounded by the triazole and terminal acetylene units thus providing catalytically active coatings.

To confirm that  $\beta$ -CD was acting as a phase transfer catalyst by making a host–guest complex with the aromatic ring of azide/acetylene compounds, a click reaction using non-aromatic compounds

#### Table 3

Click reactions of azides and acetylenes conducted in a continuous process using micro reactor based on glass micro-chip functionalized with β-cyclodextrin (5)

Entry	Azide	Alkyne	Product	Yield (%)
1	N <sub>3</sub>			99
2	(-)N <sub>3</sub>	ОН		37
3	N <sub>3</sub>	ОН		99
4	N <sub>3</sub>			99
5	N <sub>3</sub>			98
6	N <sub>3</sub>	OH		99
7	Ng Ng			99
8				98
9	N3			97
10	No No			98
11	ů, Na			97



**Figure 2.** Narrow-scan  $C_{1s}$  XPS spectrum of  $\beta$ -CD functionalized glass micro-chip (**5**) after 50 h use in a flow process for click reaction of benzyl azide and phenyl acetylene.

was also conducted. For this purpose, 1-octyl azide was reacted with propargyl alcohol under the same conditions used for benzyl azide and phenyl acetylene click reaction but only 37% yield (**8**) was obtained. These results indicate that the higher yield in short time in case of click reaction of benzyl azide and phenyl acetylene was obtained mainly due to host–guest inclusion complexation of  $\beta$ -CD and aromatic compounds and not due to any possible complexation between  $\beta$ -CD and Cu(1).<sup>10</sup> This assumption is also supported from the results of Shin et al.<sup>11</sup> who did not observe any shift in  $\beta$ -CD protons after mixing it with Cu(1) in water (D<sub>2</sub>O) while complexation of metals with terminal acetylenes is already known.<sup>15,16</sup>

To demonstrate the scope and generality of the reaction, different aromatic azides were reacted with a variety of aromatic and aliphatic acetylenes using the same micro-reactor in a continuous flow process (Table 3). All reactions show excellent yields (95–99%) indicating that the methodology can be applied for a range of azides and acetylenes.

#### Stability of modified glass surface

Stability of the modified surface is very important in flow process at industrial scale. To follow the changes that occurred on glass surface during continuous process; XPS spectrum of the modified surface was recorded after 50 h (Fig. 2, Table 1). There was almost no change in elemental composition or relative proportions of C–O or C–N/C–C bond linkages. These results indicate that modified surface remained stable and can be used for a longer time without losing efficiency.

#### Conclusions

GMC functionalized with  $\beta$ -CD can be used to develop a flow process for CuAAC click reactions. GMC surface remained stable and can be used for several cycles without any degradation or loss in efficiency. The process can be scaled up to pilot plant level using multiple GMC in parallel.

#### Experimental

#### Materials and methods

β-CD (≥97%), N,N-diisopropylethylamine (99.5%), (3-glycidoxypropyl) trimethoxysilane (98%), 5-chloro-1-pentyne (98%), dimethyl sulfoxide (DMSO, 99.9%), and copper(1) iodide (99.5%) were purchased from Sigma–Aldrich. 1-(*p*-toluenesulfonyl)-imidazole (>98%) and ethylenediamine (>98%) were from Tokyo Chemical Industry, Sodium azide was from Junsei Chemical Co., Ltd. Korea while glass micro chip ( $15 \times 45$  mm, channel length 332 mm, internal vol, 6 µL) and chip holders (Fluidic Connect 4515) were purchased from Micronit, The Netherlands.

Water was purified using Direct-Q Millipore water purification system from SAM WOO S&T Co., Ltd. Korea. Solvents used for column chromatography were distilled while other chemicals were used without further purification. NMR spectra were recorded on a Bruker AMX spectrometer at 500 MHz. Thin layer chromatography (TLC) was carried out on Merck Kieselgel 60 F254 plates. XPS spectra were recorded by using a Sigma Probe (ThermoVG, UK) photoelectron spectrometer. High-resolution spectra were obtained using monochromatic Al- $K_{\alpha}$  X-ray radiation at 15 kV and 100 W and an analyzer pass energy of 50 eV (1.0 eV step size) for wide-scan and 20 eV (0.1 eV step size) for narrow-scan. All highresolution spectra were corrected with a linear background before fitting.

Mono-6-(*p*-toluenesulfonyl)-6-deoxy-cyclodextrin ( $\beta$ -CD-OTs) was synthesized from  $\beta$ -CD as described in<sup>17</sup> which in next step was converted into mono-6-azido- $\beta$ -cyclodextrin ( $\beta$ -CD-N<sub>3</sub>) according to the procedure described in.<sup>18</sup> <sup>1</sup>H NMR data of products was in accordance with<sup>17,18</sup> (detailed procedure is given in electronic Supplementary data).

#### Modification of glass surface with β-CD

To modify inner micro-channels, GMC (1) was washed and etched as described in.<sup>19</sup> Immediately after cleaning, GMC was transferred into degassed (3-glycidoxypropyl)trimethoxysilane in a glass reaction cell. To remove trace amounts of oxygen that might enter during sample transfer, the reaction cell was filled with argon followed by vacuum and this cycle was repeated 3 times. Finally, the glass cell was refilled with argon and heated in an oil bath for 16 h at 130 °C (Scheme 1). The samples were removed from the glass cell and sonicated in acetone for 5 min, rinsed several times with acetone and *n*-hexane and dried in a stream of argon. Subsequently, the epoxide coated GMC (2) was dipped in the neat ethylenediamine as described in.<sup>14</sup> Reaction was carried out at 40 °C for 24 h followed by washing and drying steps. To attach terminal alkyne group, GMC functionalized with amine terminated monolayer (3) was dipped in a mixture of 5-chloro-1-pentyne (5 mL) and triethylamine (0.5 mL) in dry dichloromethane (5 mL) at room temperature for 24 h.

In the last step of this reaction, mono-6-azido- $\beta$ -cyclodextrin ( $\beta$ -CD-N<sub>3</sub>) was prepared according to the procedure described in<sup>17,18</sup> and clicked with acetylene terminated mono layer on GMC (**4**). For this purpose,  $\beta$ -CD-N<sub>3</sub> (500 mg, 0.43 mmol) was dissolved in DMSO/H<sub>2</sub>O (8:1, 10 mL) followed by addition of *N*,*N*-diisopropylethylamine (167 mg, 3.35 g, 1.3 mmol, 3 equiv/azide group) and Cul (20.5 mg, 0.1 mmol, 0.25 equiv/azide group). GMC functionalized with terminal alkynyl group (**4**) was incubated in the reaction mixture at 40 °C for 24 h under N<sub>2</sub> followed by cleaning steps.

To prepare the modified surface having the triazole ring (**6**) but not  $\beta$ -CD, benzyl azide was reacted with acetylene terminated surface (**4**) following the procedure described for click reaction of  $\beta$ -CD-N<sub>3</sub>.

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#### Supplementary data

Supplementary data (Synthesis of mono-6-(p-toluenesulfonyl)-6-deoxy-cyclodextrin (β-CD-OTs), mono-6-azido-β-cyclodextrin (β-CD-N<sub>3</sub>), NMR data for compounds **7–17** and XPS spectra) associated with this article can be found, in the online version, at http:// dx.doi.org/10.1016/j.tetlet.2013.04.042.

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