



# Versatile ene-thiol photoclick reaction for preparation of multimodal monolithic silica capillary columns

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## ABSTRACT

This paper presents a photografting process of monolithic silica capillary columns based on the ene-thiol click chemistry. This study is performed on a “generic” vinyl-functionalized silica monolith ( $H_{\min} 6 \pm 1 \mu\text{m}$ ). The photoclick reaction is investigated using different thiol monomers (octadecanethiol, cysteine and sodium mercaptoethanesulfonate) to prepare capillary columns dedicated to various chromatographic modes (reversed-phase, HILIC and strong cation exchange). Whatever the monomer used, the photografting reaction is achieved in less than 5 min with a relatively high thiol monomer content. This allows preparing highly retentive and efficient monolithic columns while avoiding polymerization and/or column clogging. In addition to the aforementioned properties (duration, versatility, efficiency), this photo-triggered chemical reaction allows addressing several appropriate surface functionalizations inside a single monolithic column in order to prepare nanovolume multimodal capillary columns. A multimodal biphasic monolithic column with a 1 cm length cation-exchange segment followed by a 9 cm length reversed-phase segment (SCX-RP) is prepared through two successive photografting reactions using a UV-mask to localize the reactions. This multimodal biphasic column is investigated using a model sample for the selective fractionation and separation of cationic and neutral compounds and is applied to the on-line preconcentration and separation of  $\beta$ -blockers.

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## 1. Introduction

The functionalization of silica monoliths in tiny capillary tubing is not a trivial task. Although silanization is the “gold” standard procedure to chemically modify silica stationary phases, this approach is not suitable for the preparation of multimodal chromatographic capillary columns (MCC) with several sequential zones of different surface chemistries. Multimodal chromatography, with so called biphasic (or even multiphasic) columns, is mainly devoted to the analysis of complex biological samples and its use in miniaturized systems (capillary columns for example) is challenging for detection limits and sample volume concerns. It has found applications for the analysis of small molecules as reviewed recently by Mansour [1] as well as in proteomics. For example, several articles highlight the potential of MCC for shotgun analysis in a so-called MudPIT (Multi Dimensional Protein Identification Technology) approach [2–5]. If MudPIT with packed columns has already been proven robust for the resolution of complex mixtures of

peptides, improvements in the resolution are always desirable to provide increased protein coverage. As reported by the group of Yates, such improvements could be achieved, with UltraHigh Pressure MudPIT (UHP-MudPIT) [6], by increasing the length of the C18 segment and reducing the particle size of the resin used. The main limitation to the development of such long sequential multimodal silica capillary columns (MCC) is due to the way of preparing miniaturized analytical systems integrating several zones of different surface chemistries. For example, packing different successive beds of functionalized silica particles in capillary tubing is cumbersome and labour-intensive although it has been already successfully implemented for the MudPIT approach. In addition, a lower permeability is obtained with sub-3  $\mu\text{m}$  silica particles (usually used in the reversed phase segment) compared to the one obtained with monolithic beds, which limits the capillary length i.e. the maximal efficiency attainable for the reversed phase separation consecutive to the ion-exchange fractionation. In addition, these assemblies require frits that are usually source of undesirable adsorption as well as flow inhomogeneities. On the other hand, silica monoliths are easily *in-situ* synthesized in capillary. However, the synthesis of different silica monolithic segments is difficult to control for mass production purpose. Recent works based on photochemical processes have been implemented in order to address this

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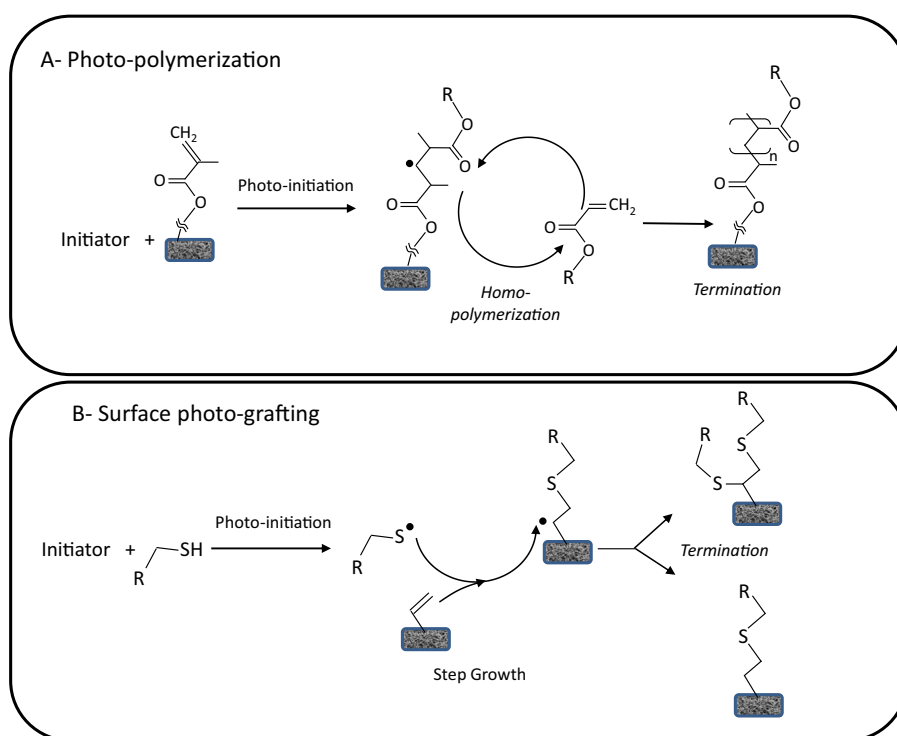
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issue with varying degrees of success [7–10]. These strategies use well-known free radical polymerization reactions of acrylate-based monomers initiated by UV-irradiation. These photopolymerization processes are rapid, efficient and versatile due to the wide variety of commercially available functional monomers. The chromatographic performances of these columns are quite satisfactory in terms of retention, but sometimes disappointing in terms of efficiency. Indeed, when the polymerization process is not properly controlled it may lead to the growth of thick layers and losses of efficiency when mesopores are sterically hindered [7]. It can also lead to column clogging when polymerization affects macropores [8].

In order to avoid this drawback while taking advantage of the rapid, simple and versatile nature of photochemical reactions, a photografting (and not photopolymerization) process is investigated. This functionalization pathway is based on the ene-thiol photoclick reaction. Compared to the free radical polymerization (Fig. 1) the photo-click reaction takes advantage of the site specific activation of thiol monomers in solution by an H-abstrating photoinitiator. The thiyl radical attacks the vinyl group tethered to the silica surface leading to a stable thioether link. Because one vinyl group is only able to react with two thiol monomers, this reaction is considered as a grafting reaction and not as a polymerization one. We have already investigated the elaboration of functionalized silica monolithic columns using commercially available octadecanethiol (C18) [11]. The as-synthesized C18 photoclicked monolithic columns present slightly higher retention properties than standard ODS monolithic columns [9]. These retention properties are close to those achieved after the polymerization or photopolymerization of lauryl acrylate onto methacrylate functionalized silica monoliths [9,12], such polymerization processes being intended to lead to highly retentive stationary phases [12].

The retentive properties of the ODT photoclicked columns can be attributed to dense layers. In addition, the value of steric selectivity (o-terphenyl/triphenylene selectivity) [13] is similar to the one measured for ODS columns. It confirms the monolayer like structure of the photografted layer, while photopolymerized layers behave differently due to the 3D-network of the polymerized layer. It was also demonstrated that the kinetic performances of the starting silica material was maintained after the functionalization process which avoided any clogging of mesopores.

Herein, we investigate the photografting of different commercial thiol monomers. Although the ene-thiol click reaction approach has been already reported for the functionalization of silica, the process involved is time consuming and not localizable [10,14–20]. The grafting of cysteine and sodium mercaptoethanesulfonate (SMES) onto a vinyl activated silica monolith is followed and characterized in hydrophilic interaction (HILIC) and strong cation exchange (SCX) chromatography respectively. Retention factors, selectivities and ion-exchange capacities are compared with the values reported in the literature for silica monoliths modified either with the classical silanization pathway or with polymerization. It is also verified that the kinetic performances of the starting silica materials are maintained, thus confirming that the functionalization process avoids clogging of the mesopores. To illustrate the benefit of the presented photochemical approach at the capillary format, multimodal biphasic (SCX-RPLC) chromatographic capillary columns are prepared. Their potential is illustrated through two examples of applications: (i) the selective fractionation and separation of neutral and charged model solutes, (ii) the preconcentration of cationic  $\beta$ -blockers in cation exchange mode followed by their separation by reversed-phase chromatography.



**Fig. 1.** Schematic representation of the photopolymerization and photografting processes. Photopolymerization is based on the free radical polymerization of acrylate and methacrylate monomers. It should be noticed that the same reaction occurs between monomers in solution leading to the formation of polymers in solution responsible for an increase of its viscosity. At the opposite, photografting is based on the selective activation of thiol monomers in solution which react with vinyl groups tethered to the surface of the silica monolith. This process leads only to dimers in solution if two thiyl radicals react together. The substituent R is chosen to bring the desired surface retention properties (i.e. C12 or C18 alkyl chains, zwitterionic or charged groups).

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