



Photopolymerization of acrylamide as a new functionalization way of silica monoliths for hydrophilic interaction chromatography and coated silica capillaries for capillary electrophoresis



R. El-Debs¹, A. Marechal¹, V. Dugas, C. Demesmay*

Université de Lyon, Université Claude Bernard Lyon I, Institut des Sciences Analytiques, UMR CNRS 5280, 5, rue de la DOUA, 69100 Villeurbanne, France

ARTICLE INFO

Article history:

Received 20 September 2013

Received in revised form 9 December 2013

Accepted 10 December 2013

Available online 24 December 2013

Keywords:

Monolith

Photopolymerization

Surface functionalization

Acrylamide

HILIC

μSPE

ABSTRACT

A simple, rapid and localizable photochemical process for the preparation of hydrophilic coated capillary and silica-based monolithic capillary columns is described. The process involves the free radical polymerization of acrylamide monomers onto acrylate pre-activated silica surface triggered by UV photoinitiation. The experimental conditions (monomer content, time of irradiation) were optimized on silica monolithic columns by monitoring the evolution of the chromatographic properties (retention, permeability, efficiency) in HILIC mode using a set of nucleosides as test solutes. Compared to thermal polymerization process, the photoinitiation allows the preparation of highly retentive and efficient HILIC monolithic columns in less than 10 min of irradiation. This process was then successfully applied to the surface coating of fused silica capillary walls. In addition to its relative high stability and ability to reduce the electroosmotic flow, this polyacrylamide coating is localizable. Benefits of this localizable photochemical process are highlighted through the conception of an in-line integrated bimodal microseparation tool combining a SPE preconcentration step on a photografted silica monolith and an electrokinetic separation step in a polyacrylamide photopolymerized capillary section. Two neuropeptides are used as model solutes to illustrate the suitability of this approach.

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1. Introduction

The miniaturization of separation systems is a major advance in the field of analytical chemistry. Indeed, it brings solutions to different issues such as small sample volume handling, reduction of solvent consumption and/or expensive reagents while allowing direct integration of successive steps (sample preparation, separation, labeling and detection) for automation. In the toolbox of analytical scientists, a wide variety of tools rely on the use of solid stationary phases. Integration of solid phases inside miniaturized devices is thus challenging. One of the most convenient ways to insert solid stationary phases into miniaturized columns (capillary tube, microfluidic channels) relies on the in situ synthesis of porous materials such as organic or inorganic monoliths. If photopolymerized organic monoliths are easily in situ synthesized from a large variety of monomers covering a wide range of interaction mechanisms, they present some specific drawbacks. Compared to their inorganic counterparts (silica monoliths) their permeability is lower as well as their efficiency when used in hydrodynamic mode [1–3] and their mass transfer kinetics is slower. Moreover,

organic monoliths are very sensitive to organic solvents effects and particularly in highly aqueous and methanolic solvent in which their performances deteriorate [2]. Although their synthesis may not be localized as for their organic counterparts, inorganic monoliths like silica monoliths, may also be in situ synthesized according to a sol-gel process and do not present these specific drawbacks. However, at the end of their more time-consuming synthesis that leads generally to bare silica monoliths, their surface chemistry has to be subsequently modified. Such surface modifications can be realized in different ways as reported by Tanaka and colleagues [4]. The classical way to functionalize silica monoliths, silanization [5–8], is time consuming. Besides this classical approach, some authors [9] have proposed less-conventional surface functionalization methods of silica or hybrid silica monoliths such as thermal polymerization or hydrosilylation of hybrid allyl silica monoliths [10,11], thermally initiated free radical polymerization [12], nucleophilic substitution of amino-modified monoliths [13], oxidation of mercapto moieties into sulfonic acid groups [14]. However, all these functionalizations are thermal and time-consuming processes. Moreover, if the integration of several steps with different surface chemistries in a miniaturized system is required, these approaches are not adapted due to the inability to localize the grafted area. Thus, photopolymerization, a localizable functionalization process, is particularly suited to meet this

* Corresponding author. Tel.: +33 0437423552.

¹ These authors contributed equally to this work.

objective. Moreover, the versatility of photopolymerization as a functionalization process for silica monoliths has already been demonstrated in our previous work [15], with the elaboration of highly efficient monolithic columns for reversed phase and anion exchange modes. The two-step process involves, in a first step, the derivatization of the overall silica surface (unlocalized) with [γ -(methacryloyloxy)propyl]trimethoxysilane (γ -MAPS) to create a layer of acrylate photoreactive groups. Appropriate surface functionalities are then introduced in a second step, through localized surface initiated photopolymerization. In this work, we demonstrate the efficiency of photopolymerization through the functionalization of different silica supports (monoliths and fused silica capillaries) with acrylamide, as stationary phase for the HILIC mode and as covalent coating in order to reduce electroosmotic flow and prevent adsorption in capillary electrophoresis. In fact, the HILIC separation mode is currently drawing a lot of interest due to the possibility to separate small polar compounds on polar stationary phases. Compounds are shared between a water-enriched layer partially immobilized on the hydrophilic stationary phase and the less polar mobile phase. The elution order is so inverted compared to RPLC. Moreover, the HILIC mode allows solving many previously difficult separation problems, such as the separation of small organic acids, basic drugs, and other neutral and charged substances. It has been successfully applied to the analysis of carbohydrates, peptides, sugars and polar pharmaceuticals [16–24]. Due to its polar amide functions, acrylamide is particularly adapted for HILIC mode. In fact, this polymer has been already grafted on different silica supports such as silica particles (commercial column [25]) or silica monoliths [26] and has been recently introduced for the preparation of an organic-silica hybrid monolithic column [27]. However, the grafting is always performed according to a thermal process and is time consuming.

Here, we propose the photopolymerization as a versatile, localizable and one-step surface functionalization to graft polyacrylamide (PA) on pre-functionalized silica monolith in a few minutes. Photopolymerization has been first optimized and prepared HILIC monolithic columns have been compared to other HILIC columns grafted with acrylamide in order to evaluate the potential of photopolymerization. Thereafter, photopolymerization of acrylamide has been implemented for the covalent coating of fused silica capillaries. The acrylamide coating was first introduced by S. Hjertén in 1985 to eliminate electroendosmosis and adsorption of solutes [28] responsible for zone distortion loss in resolution. Electroosmotic mobility values as low as 10^{-5} cm²/V s (at pH 10) were obtained with this coating [29] or with similar ones: dimethylacrylamide coating [30] or cross-linked polyacrylamide [31]. Although this coating has found various applications in capillary electrophoresis for the separation of several classes of compounds [30,32–37], its synthesis according a classical thermal polymerization requires numerous hours depending on conditions [30,32,33]. Moreover, the acrylamide coating is hung on the entire surface of the capillary and cannot be localized in a defined zone if necessary. In this work, we propose the use of photopolymerization to locally coat the surface of fused silica capillaries with acrylamide in only 10 min. The repeatability, reproducibility and stability of the coating are here demonstrated. At last, the interest of localized photopolymerization is here demonstrated with the on-line coupling of a preconcentration step (implemented on a silica monolith photografted with C12 alkyl chains) and an electrokinetic separation step (implemented in an empty section of a fused silica capillary photografted with acrylamide for the analysis of two neuropeptides): Met-enkephalin and LEU-enkephalin. Indeed, the development of in-line SPE preconcentration strategies coupled with electrokinetic separations has attracted an increased interest in the last few years [38] to automate the analysis of small sample volumes, at low concentrations and with less handling. One of the

key features to success in this development is to be able to control the surface chemistries of both preconcentration and separation zones in order to be able to optimize both steps independently. In the chosen example, the combination of a C12 monolithic stationary phase will allow the preconcentration of the peptides in the reversed mode, whereas the acrylamide one will suppress the electroosmotic flow to increase resolution in capillary zone electrophoresis as will be demonstrated.

2. Experimental

2.1. Reagents and chemicals

All the reagents used were of analytical grade. Tetramethoxysilane (TMOS), methyltrimethoxysilane (MTMS), urea, PEG (Mn = 10,000), lauryl acrylate, benzoin methyl ether (BME), triethylamine (TEA), methanol (HPLC grade), peptides MET-enkephalin and LEU-enkephalin, thiourea, naphthalene and all the nucleosides were purchased from Sigma-Aldrich (France). Acetonitrile (ACN) (HPLC grade) was purchased from VWR. Acetic acid was from Riedel-de Haënn (Germany). [γ -(methacryloyloxy)propyl]trimethoxysilane (γ -MAPS) and acrylamide were purchased from Acros (France). Tetrahydrofuran (THF) was from Rathburn (UK). Propylbenzene, pentylbenzene, phenylheptane, and octylbenzene were purchased from Fluka (Switzerland). Hexylbenzene was from Alfa-Aesar (France). Phosphate buffer 20 mM and 10 mM were prepared using the adequate proportions of KH₂PO₄ and K₂HPO₄ in water (all reagents were from Sigma-Aldrich, France). All aqueous solutions were prepared using >18 MDI water (Millipore, Molsheim, France). PTFE-coated fused-silica capillaries (TSU 75 μ m i.d.) were purchased from Polymicro Technologies (USA).

2.2. Preparation of methacrylate pre-functionalized silica supports

2.2.1. Synthesis of silica monolith

The preparation conditions of silica monolithic columns were similar to those reported by Hara et al. [39]. Monolithic silica capillary columns were prepared from a mixture of TMOS/MTMS (85:15, v/v). A total of 18 mL of this mixture was added to 40 mL acetic acid (0.01 M) containing 1.9 g PEG and 4.05 g urea and stirred at 0 °C for 30 min. Then the temperature was raised up to 40 °C and the mixture was stirred for 10 min before it was charged into fused-silica capillaries. Filling steps of capillary columns were carried under nitrogen (N₂) pressure using a Nanobaume system (CIL-Cluzeau Info Labo SA). After that, the filled and blocked capillaries were kept at 40 °C overnight for gelification and aging. Then, the temperature was raised slowly up to 120 °C and maintained at this temperature for 4 additional hours to form mesopores with the ammonia generated by the hydrolysis of urea. The monolithic silica columns were then cooled and washed with methanol. Morphological characterization of the monoliths was done using a JEOL nanoscope JCM 5000 (Japan) scanning electron microscope after a gold coating of the samples. The hydrodynamic permeability was determined using Darcy's law and naphthalene as t₀ marker (nonretained solute) in 95/5 acetonitrile/water mobile phase.

2.2.2. Methacrylate pre-functionalization

Prior to surface photopolymerization, silica monoliths or fused silica capillary surfaces were derivatized with a methacrylate functionalized silane (γ -MAPS) to anchor acrylamide coating on the surface by covalent bonding. The silanization solution was composed of the γ -MAPS silanizing agent (5%, v/v), a catalyst (triethylamine) (2.5%, v/v), and a solvent (95/5, v/v, MeOH/Water). Silanization was performed at 80 °C during 3 h under hydrodynamic

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