In-situ photopolymerized polyhedral oligomeric silsesquioxane-derived monolithic capillary columns with quinidine functionality for enantioseparation by nano-liquid chromatography

The successful fabrication of monolithic capillary columns for enantiomer separations was achieved within vinylized fused silica capillaries via fast “one-pot” photo-initiated free radical polymerization reaction. A mixture consisting of polyhedral oligomeric silsesquioxane, O-[2-(methacryloyloxy)ethylcarbamoyl]-10,11-dihydroquinidine was copolymerized in the presence of n-butanol, ethylene glycol and photo-initiator 2,2-dimethoxy-2-phenylacetophenone. The morphology of the resultant polymer hybrid inorganic-organic material and its permeability as well as porosity can be controlled by adjusting the composition of the monomers and binary porogenic solvent. The chromatographic characteristics of the columns have been investigated. Separation factors of N-acetyl-phenylalanine (Ac-Phe) and dichlorprop dropped with decrease of chiral functional monomer. Permeability was better when the macroporogen ethyleneglycol was present at higher concentrations during the polymerization. In general, the chiral compounds were well separated (dichlorprop: \( \alpha = 1.53, R_s \) up to 4.14; Ac-Phe: \( \alpha = 1.36, R_s \) up to 2.69) by nano-HPLC with an optimized enantioselective monolithic capillary column which can be prepared within a few minutes.

Keywords:
Chiral stationary phase / Enantiomer separation / Inorganic-organic hybrid monolith / Nano-HPLC / Photopolymerization DOI 10.1002/elps.201900316

Additional supporting information may be found online in the Supporting Information section at the end of the article.

1 Introduction

Monolithic materials as chromatographic column beds exhibit several advantages over particulate beds such as high permeability owing to the macroporous structure, flexible tailoring of the surface chemistry by simple copolymerization of various functional monomers, and a fast capillary column fabrication [1]. In the late 1980s, monolithic polymers based on polystyrene, polymethacrylate, and polyacrylamide were first introduced by Hjerten and Svec as new HPLC media for fast separation of small molecules and macromolecules [2–4]. Another popular monolith approach is based on polyhedral oligomeric silsesquioxane (POSS) with cage-like structure of inorganic silicon and oxygen [1]. It was used for fabrication of a variety of new POSS-based hybrid inorganic-organic polymers. Methacryl-substituted (POSS-MA) [5–8], acrylopropyl-POSS [9], vinyl-POSS [10, 11], octakis-glycidyldimethylsilyl-POSS [12], and octakis(3-mercaptopropyl)octasilsesquioxane-POSS [13] were reported for monolithic column preparation. Free radical copolymerization [7], thiol-based click [5, 7], and ring-opening polymerization [14] are the three most commonly used methods to prepare POSS-based monoliths.

There is a need for chirally functionalized materials which can cope with the requirements of chiral stationary
phases for enantioselective separation of a wide spectrum of chiral compounds by HPLC [15–19]. Quinine (QN) and quindine (QD) carbamate derivatives have shown great potential as chiral selectors; they have been immobilized on silica particles or monoliths, or were incorporated into polymeric matrices for enantiomer separations of chiral acids [20–29]. What monoliths is concerned, besides direct in-situ photoinitiated and thermally initiated copolymerization of methacryl-functionalized QN and QD carbamates [21–23, 30, 31], post-modification strategies have also been pursued [32]. Wolter et al. developed a functionalized monolithic polysiloxane-poly(alkyl acrylate) composite monolithic column using thiol-ene click reaction for enantioseparation by nano-HPLC [28]. In this polymerization reaction, the chiral selector, QN carbamate, was used as the functional monomer. Further, thiol-click immobilization of phenyl carbamate of cinchonidine in a monolithic structure was also demonstrated [13].

In our present study, POSS-based monoliths with pendant QD carbamate moieties are synthesized in situ in UV-transparent fused-silica capillaries via fast “one-pot” photo-initiated radical polymerization reaction. POSS-MA, chiral monomer O-2-(methacryloyloxy)ethylcarbamoyl]-10,11-dihydroquinidine (MQD) are copolymerized in presence of a binary porogenic solvent mixture (n-butanol and ethylene glycol) and photo-initiator 2,2-dimethoxy-2-phenylacetophenone (DMPA). Detailed investigations on the effect of the constituents in the polymerization mixture such as POSS-MA, chiral monomer MQD, and binary porogen were carried out. The goal was to optimize both the permeability, pressure resistance, enantioselectivity, and resolution of the monolithic capillary columns. Helium ion microscopy (HIM) images were generated for the characterization of the morphology of the monolithic materials. On-capillary fluorescence measurement was applied to confirm the incorporation of functional monomer MQD in the polymer structure. The chromatographic characteristics of all columns were systematically studied by nano-HPLC. Parameters like backpressure, permeability, porosity, and enantioselectivity were evaluated. Some mobile phase parameters such as buffer additives were explored as well. Furthermore, run-to-run separation repeatability and batch-to-batch reproducibility were confirmed.

2 Materials and methods

2.1 Chemicals and materials

The chiral monomer MQD was synthesized as described elsewhere [21]. Methacryl-substituted POSS-MA with a cage-like nanostructure, cage mixture, n = 8,10,12, was purchased from Sigma-Aldrich (Munich, Germany). 3-Trimethoxysilylpropylmethacrylate (TMSPM) employed as the silanization reagent for the fused-silica wall was supplied by Sigma-Aldrich. The porogenic reagents, n-butanol and ethylene glycol as well as the photo-initiator for the polymerization DMPA were supplied by Sigma-Aldrich.

First, the internal surface of the bare fused silica capillary was vinylized with TMSPM reagent through silanization reaction [33]. In brief, the bare fused silica capillary was firstly filled with 1 M NaOH and allowed to react for 2 h at room temperature. Afterwards, the capillary was rinsed with 1 mL deionized water and then with 1 mL toluene at the flow rate of 2 µL/min. Subsequently, the mixture of TMSPM and toluene (40/60, v/v) was filled into the capillary with a flow rate of 2 µL/min. The capillary was sealed with rubber stoppers at both ends and incubated in a water bath at 60°C for 20 h. Finally, the internal surface of the capillary was dried with nitrogen at room temperature.

The POSS-MQD hybrid monolith was prepared via an in situ copolymerization strategy (Fig. 1). The porogen solution consisted of ethylene glycol as macro-porogen and n-butanol as micro-porogen. The porogen mixture was homogeneously mixed by sonication for 1 min in the ice/water bath. Afterwards a portion of porogen mixture was used for dissolving an amount of 7.5 mg DMPA and the remaining portion was used for dissolving the chiral monomer MQD (61 mg) and the crosslinker POSS-MA (122 mg). In order to investigate the effect of porogen on the chromatographic performance of the resulting monolith, the ratio of macro- and microporogen was varied in the range of 1:6, 1:5, and 1:4 v/v. The detailed compositions of the polymerization mixtures are summarized in Table 1. Then, these chemicals were mixed together in the darkness to obtain the prepolymerization mixture. The resultant solutions of these reaction mixtures were purged with nitrogen for 2 min to remove the oxygen in the mixture. The prepolymerization solution was further infused into the vinyIized capillary with a syringe pump at a flow rate of 75 µL/h. Afterwards, the filled capillary was sealed with rubber pieces and fixed onto a rotating table with the speed of 60 cycles per min. The in-situ polymerization reaction was subsequently initiated under the UV lamp for 15 min at room temperature. The distance between the UV lamp (OSRAM Ultra Vitalux 300 W 230 V E27 as the UV source) and the capillary was 10 cm. After reaction, the capillaries were flushed with methanol overnight for removal of residual monomers and porogen, and then were stored in methanol until usage.

Distilled water used in all experiments was purified by Elga PURELAB Ultra Water Purification System (Celle, Germany). Acetic acid (HAc), ammonium acetate (NH₄Ac), sodium hydroxide (NaOH) and toluene used in the silanization process were obtained from Sigma-Aldrich. HPLC-grade methanol was supplied by Sigma-Aldrich, HPLC-grade acetonitrile was purchased from JT Baker Chemical (Deventer, The Netherlands).

The UV-transparent fused silica capillary columns with 100 µm i.d. × 360 µm o.d. were purchased from Polymicro Technologies Phoenix, AZ USA.

2.2 Preparation of monolithic capillary columns


2.3 Characterization with HIM and fluorescence measurement

Scanning HIM images were used to characterize the monolithic materials by using the Helium/Neon Ion Microscope ORION NanoFab (Carl Zeiss, Peabody, MA). The monolithic column was cut to 5 mm length and then put perpendicularly on the sample holder. The samples were sputtered with a thin layer of AuPd (approx. 40 nm) and imaged using the flood gun for charge compensation. Cross sectional views of the monolithic materials in the capillary were obtained with different magnifications.

Fluorescence measurements were used to characterize the successful incorporation of MQD selector into the monolithic polymer.

2.4 Permeability test

The back pressure of the monolithic columns was measured with methanol as the mobile phase by varying the flow rate of the system. The permeability $K$ can be calculated according to Darcy’s law (Eq. 1)

$$K = \frac{F \cdot \eta \cdot L}{\pi \cdot r^2 \cdot \Delta P}$$

where, $F$ represents the volumetric flow rate, $\eta$ is the viscosity of the mobile phase, $L$ and $r$ represent the column length and the inner radius of the column, respectively, and $\Delta P$ is the drop of column back pressure. The backpressures of the monolithic column were corrected by subtraction of the system back pressure.

2.5 Chromatographic characterization of monolithic capillary columns by nano-HPLC

Racemic test samples N-acetyl-phenylalanine (Ac-Phe) and dichlorprop, dissolved in methanol at 1 mg/mL, were used for characterization of the enantiorecognition capability of the monoliths.

Nano-HPLC experiments were performed on a Dionex Ultimate 3000 RS LC Nano System (Thermo Fisher Scientific, Idstein, Germany). For chiral separation tests on POSS-MQD hybrid monoliths, mobile phase A consisted of methanol and mobile phase B of methanol, acetic acid and ammonium acetate (98:2:0.5, v/v/w). For the investigation of ion-strength effects on the chiral separation, mobile phases with 2.5, 10, 25, 50, 75, and 100% B were adjusted. Acetone was used as void volume marker. The injection volume was 0.1 µL. All the separations were performed at 25°C. UV detection was performed at the wavelength of 280 nm for dichlorprop and 214 nm for Ac-Phe.

3 Result and discussion

3.1 Choice of materials and fabrication of the monolithic capillary columns

MQD was used as the chiral selector for the enantiomer separation. The selector provides several interaction sites for intermolecular interaction with chiral compounds, (i) the protonated nitrogen of the quinuclidine ring as weak anion exchanger for electrostatic interaction (ion pairing) with acidic analytes, (ii) a carbamate group with hydrogen donor and acceptor moieties for intermolecular hydrogen bonding with complementary sites of the analytes, (iii) the quinoline ring for intermolecular $\pi-\pi$ interaction, and (iv) the quinoline and quinuclidine group are steric residues for van der Waals type dispersive interactions or steric repulsion with the analytes or their residues (see Supporting Information Fig. S1). POSS was selected as crosslinker and basic monomer forming the monolith backbone. Through its multiple polymerizable groups it can produce a rigid...
The polymerization and formation of the porous structure starts from a solution of the monomers in porogenic solvents. After initiation of the polymerization by UV irradiation, the radical addition reaction proceeds until onset of phase separation due to insufficient thermodynamic solubility of the growing polymer chains in the porogenic solvents. However, polymerization then proceeds in the precipitated polymer nuclei, which are swollen with monomer and further grow to the final structure. The porogenic system in the reaction mixture thus shows a significant impact on the formation of the monolith morphology [34]. Ethylene glycol and n-butanol are widely reported porogens for the preparation of POSS-based hybrid monoliths [7, 35]. This binary porogen was used by considering the solubility of the monomer, POSS-MA and QD derivative. Ethylene glycol, represented the macro-porogen in this polymerization; increasing the amount of ethylene glycol led to the larger pores in the polymer. On the contrary, increasing the ratio of micro-porogen, n-butanol, resulted in a decrease of the pore sizes. The mechanism of the polymerization reaction was based on conventional free radical copolymerization.

To investigate the impact of the content of each reactant on the stability and permeability of the monolithic column, seven columns were synthesized. The constituents of the pre-polymerization mixtures, ratio of porogens, permeability, and total porosity of the resultant monolithic columns are summarized in Table 1.

The incorporation of the chiral selector in the monolith structure was proven by on-capillary fluorescence measurement through the UV transparent coating of the capillary columns based on the native fluorescence of MQD. It can be seen from Supporting Information Fig. S2a that monolith 1 shows fluorescence emission upon light irradiation at the wavelength of 358 nm. The blue fluorescence demonstrates the successful incorporation of the chiral selector in the monolithic bed.

### 3.2 Permeability test

Back pressures for each monolith were measured at different flow rates with methanol to evaluate the mechanical stability and column permeability. The relationship between backpressure and volumetric flow rate for these columns is presented in Figure 2. The good linearity of these curves demonstrates that no appreciable deformations on the framework of the monolithic material occurred in the linear range. Therefore, desirable mechanical stability was obtained for all columns.

From these experiments, the permeability of each column was calculated based on Eq. (1) (Darcy’s law). The results are summarized in Table 1 (Supporting Information Fig. S3 depicts graphically the permeability and porosity of the different columns). It can be seen that the permeability decreased (from $0.87 \times 10^{-14}$ m$^2$ to $0.22 \times 10^{-14}$ m$^2$) with
increasing ratio of n-butanol/ethylene glycol, which means that a higher content of ethylene glycol in the reaction mixture caused a higher permeability (cf. monoliths 1,2,3). As mentioned above, ethylene glycol can be considered as the macro-porogen in the polymerization; higher contents induce the phase separation earlier in the polymerization process which resulted in a high percentage of (larger) macro-pores in the monolith. When the monomer-to-porogen ratio was decreased a higher porosity and increased permeability was obtained, as expected (cf. monoliths 2,4,5). It is also worthwhile noting that upon decrease of the chiral monomer content the permeability increased (cf. monoliths 3,6,7).

3.3 Characterization of morphology by HIM

The monolithic matrix was formed by co-polymerization in presence of porogens based on the mechanism of conventional free radical copolymerization in accordance to a nucleation and growth mechanism. For this reason, a microglobular structure as common for such pore formation approach can be observed in the HIM images (Fig. 3).

The HIM images clearly show that the monolithic matrices were well attached onto the inner walls of the fused-silica capillaries. In addition, the reticular skeletons with the macro though-pores were observed for all the monoliths. Of all the columns, monolith 2 exhibited the most homogenous polymer morphology and was therefore selected as optimized material for further chromatographic tests (vide infra). Monoliths 5,6,7 showed stronger irregularities (Fig. 3C–E) and were therefore not considered further. As can be seen from Fig. 3, the morphology of the skeleton structure can be adjusted by the composition of the prepolymerization mixture through experimental variables such as content of functional monomer MQD, crosslinker POSS-MA, and porogens.

In our study, the effect of different ratios of porogen and POSS-MA on the morphological structure of the monolithic columns (monoliths 2, 4, and 5) was investigated. From Fig. 3A, B, and C, a larger macropore size can be deduced when higher ratios of porogen in the prepolymerization solution were used. Correspondingly, microglobules with smaller size were generated because of the low ratio of POSS-MA used in the reaction mixture. Furthermore, with increase of the pore size calculated porosities increased as well from 78 to 95% (see Table 1).

Figure 3D (monolith 6) and E (monolith 7) reflect the effect of the amount of the chiral monomer MQD in the polymerization mixture. In spite of different amounts of chiral monomer MQD in the polymerization mixture, similar microglobular polymer structure with comparable skeleton size can be observed; it seems that POSS-MA and porogens, which were both kept constant in the prepolymerization mixture are the dominant factors for the formation of the polymer morphology.

Figure 2. Backpressure versus flow rate curves of all the monoliths.

Figure 3. HIM photograph of monolithic columns synthesized by UV-initiated free radical polymerization, (A) column 2; (B) column 4; (C) column 5; (D) column 6; (E) column 7. Top: field of view 125 microns; Bottom: field of view 15 microns.
Figure 4. Chiral separation of dichlorprop (A) and AC-Phe (C) on monolithic column #2 with isocratic elution at variable buffer concentrations (MeOH/acetic acid/ammonium acetate 98:2:0.5, v/v/w as mobile phase used undiluted and diluted 1:1, 1:3; 1:39 with MeOH). Effect of buffer concentrations on the chromatographic parameters of dichlorprop (B) and AC-Phe (D). Monolith 2: 100 μm i.d. \( \times \) 22 cm, DAD at 280 nm for dichlorprop and 214 nm for AC-Phe. Flow rate 0.3 μl/min. Injection volume: 0.1 μl. Temperature: 25°C. Elution order: Dichlorprop, \( R < S \); AC-Phe, \( S < R \).

### 3.4 Chromatographic characterization

To evaluate the chromatographic performance of the columns, the separation of the chiral test compounds dichlorprop and Ac-Phe was tested on the monolithic capillary columns by Nano-HPLC in isocratic elution mode with MeOH/acetic acid/ammonium acetate (98:2:0.5, v/v/w) as the mobile phase.

First, a screening for the most promising capillary columns was carried out (see Supporting Information Fig. S4). Monolithic capillary column 3 exhibited long run times (180 min), poor enantioselectivity and high backpressure caused by its low permeability. It was therefore not further considered. Monoliths 5, 6, and 7 showed significantly lower enantioselectivity, due to their lower selector concentration, than capillary columns 1, 2, and 4 (see Supporting Information Figs. S4 and S5). Baseline separation with good resolution for the enantiomers was achieved on monolithic columns 1, 2, and 4. In spite of faster enantiomer separations on monolithic column 1, monolithic column 2 was providing higher resolutions and considered a good compromise between speed and resolution. Overall, monolithic capillary column 2, however, was considered a good compromise between speed and resolution. Exemplary chromatograms are depicted in Fig. 4 (corresponding chromatograms for monolith 1 can be found in Supporting Information Fig. S6).

The separation for dichlorprop on the new POSS-MQD inorganic-organic hybrid monolithic column 2 \( (R_s = 3.0; \text{ at counterion (buffer) concentration of 0.4 M acetate}) \) compares well with the nano-HPLC performance on organic polymer-based monoliths with the same functional monomer incorporated in a HEMA-co-EDMA polymer matrix \( (R_s = 3.5; \text{ under hydro-organic conditions}) \) [36]. It shows also similar resolution as a silica-based monolith with immobilized QN carbamate selector \( (R_s = 2.5; \text{ under polar organic conditions in CEC mode}) \) [31, 37]. On a commercial Chiralpak QD-AX (5 μm) similar resolution is obtained \( (R_s = 2.9) \) for dichlorprop but at shorter run times (8 min).

### 3.5 Effect of mobile phase additive concentration on the chiral separation

Isocratic polar organic elution mode was used for the investigation of the effect of the additive concentration in the mobile phase on monolithic capillary column 2. To do so, chiral separation of dichlorprop and Ac-Phe was performed by variation of the additive concentration in the mobile phase.
Table 2. Run-to-run repeatability study for column #2a, #2b and #2c under the same condition, and column-to-column (batch-to-batch) reproducibility study of these three columns

| Analyte          | Monolith #2a RSD (%) | Monolith #2b RSD (%) | Monolith #2c RSD (%) | RSD (%) |
|------------------|----------------------|----------------------|----------------------|---------|
| (R)-Dichlorprop  | 0.48                 | 0.85                 | 0.18                 | 2.11    |
| (S)-Dichlorprop  | 0.47                 | 0.55                 | 0.47                 | 1.95    |

The effect of acetate concentration C on retention factors k of dichlorprop and Ac-Phe enantiomers in polar organic elution mode follows the stoichiometric displacement model for ion-exchange (logk = logKz – Z log [C], with Z being the slope and being directly proportional to the ratio of effective charge numbers of the analyte and counterion; logKz is the intercept of this trend line) (see Supporting Information Fig. S7). The retention decrease upon increasing concentration of additive is due to the anion exchange mechanism: (i) ammonium ions are co-ions for the selector in this anion-exchange process and (ii) acetate as the displacer in the separation facilitates the analytes’ elution. When using a low buffer concentration (10.4 mM), the elution strength is weak and long run times are the result. At the same time, the improperly balanced ionic interactions at such low ionic strength conditions cause also enhanced peak broadening due to a slow (adsorption-)desorption kinetics.

### 3.6 Run-to-run and column-to-column reproducibility study

To investigate the reproducibility of the column manufacture, three new monolithic capillary columns of monolith 2 were prepared by batch-to-batch synthesis (batch a, b, and c), respectively, with the same composition of the prepolymerization mixture. For each column, the run-to-run repeatability study was tested by multiple injections of dichlorprop. Table 2 shows the good repeatability of the run-to-run test on each column with precision less than 0.85% for retention times of each peak, implying the good stability of the enantiomer separation on these columns over time. The column to column reproducibility test confirmed a good batch-to-batch reproducibility (2.11% for (R)-dichlorprop and 1.95% for (S)-dichlorprop (Supporting Information Fig. S8). Therefore, it can be concluded that the polymerization process of the columns can be well controlled by the established column preparation protocols.

### 4 Concluding remarks

This paper deals with the in-situ synthesis of enantioselective POSS-based monoliths in UV-transparent fused-silica capillaries via fast “one-pot” photo-initiated copolymerization reaction of monomer POSS-MA, chiral monomer MQD and porogen (n-butanol and ethylene glycol) in the presence of photo-initiator reagent DMPA. Synthesis conditions of the monolith, poly(MQD-co-POSS-MA), were investigated in detail by adjusting the content of POSS-MA, chiral monomer MQD and binary porogen. HIM images revealed the morphology of the monolithic polymer including the skeleton structure, microglobular structure, and homogeneity of the morphology. On-capillary fluorescence measurement documented the successful incorporation of the chiral selector MQD into the polymer materials. Subsequently, a chromatographic characterization by nano-HPLC was performed for all these columns in terms of backpressure, permeability test, and enantioselectivity on each column.

Enantioseparations were achieved by nano-HPLC with dichlorprop and N-acetyl-Phe as the model analyte. The effect of separation conditions including buffer concentration was investigated as well. The results revealed that retention can be considerably reduced with increase of counterion concentrations in the mobile phase confirming an anion-exchange process as the main retention mechanism. Highly satisfactory precision was obtained for run-to-run repeatability which demonstrates the good stability of the enantiomer separation on these columns. The good precision of enantiomer separations of dichlorprop in the batch-to-batch column preparation test indicates a well reproducible synthesis process. Due to the fast monolith synthesis by photopolymerization in a few minutes, this process of column fabrication may be of great interest for the preparation of enantioselective monolithic capillary columns.

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