



Short communication

## *In situ* sol–gel preparation of porous alumina monoliths for chromatographic separations of adenosine phosphates

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## ARTICLE INFO

## Article history:

Received 19 January 2011

Received in revised form 19 March 2011

Accepted 23 March 2011

Available online 1 April 2011

## Keywords:

Alumina

Monolith

Sol–gel

Capillary column chromatography

Adenosine phosphate derivatives

## ABSTRACT

A method enabling the *in situ* preparation of porous alumina monoliths within 100  $\mu\text{m}$  i.d. fused silica capillaries has been developed. These monoliths were prepared using the sol–gel process from a mixture consisting of an inorganic aluminum salt, a porogen, an epoxide, and a solvent. We investigated the effects of varying the preparation conditions on the physical characteristics of the monoliths with respect to their potential application in chromatographic separations. The best columns were obtained from a mixture of aluminum chloride hexahydrate, N,N-dimethylformamide, water, ethanol and propylene oxide. Adenosine phosphates were then separated in the optimized column with retention increasing according to number of phosphate functionalities.

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## 1. Introduction

The concept of the monolithic column has been introduced two decades ago as an alternative to the particle packed column for applications in high-performance liquid chromatography. Porous monoliths have been shown to have higher permeability allowing for analysis at higher flow rates therefore enabling faster analyses and high-throughput screening [1–3].

The sol–gel preparation of silica-based monolithic materials is the most widely studied approach to the inorganic monoliths, thanks to the tremendous work of Tanaka and his colleagues [4–6]. This process, which utilizes alkoxysilane precursors, offers many options in structural alterations during the preparation leading to the formation of monoliths with micrometer-sized throughpores as well as mesopores. Bonded silica-based monolithic columns have been used in a wide variety of chromatographic applications [7–12].

Recent literature indicates an increased interest in the preparation of monolithic columns from metal oxides other than silica. For example, zirconia-based monoliths have received significant attention as they exhibit certain advantages over silica including resistance to high temperatures and extreme pH. In contrast to silica, which can only be used as a cation-exchanger, the amphoteric character of other metal oxides makes them suitable both for

cation and anion exchange. An excellent review of the properties and preparation of particle-packed metal oxide stationary phases has been published by Nawrocki et al. [13,14].

One of the approaches to obtaining metal oxide-based monolithic columns is coating the surface of a well-defined porous silica monolith with a layer of metal oxide. This method has been utilized for the preparation of zirconia- [15,16] and titania-based monoliths [17]. Homogeneous coverage over the entire monolith is required in order to achieve the highest efficiency.

Direct *in situ* synthesis of metal oxide monoliths stems from alternative methods to the sol–gel process based on the use of metal alkoxide precursors  $[\text{M}(\text{OR})_n]$  [15,18–20] or the use of metal salts  $[\text{MX}_n]$  [21–26]. However, only a few reports describe preparation of these types of monolithic materials. Consequently, this area is currently not fully developed and many challenges involving preparation of highly porous and efficient stationary phases still need to be overcome.

Acid-catalyzed sequential hydrolysis and polycondensation of metal alkoxides in the presence of water-soluble porogenic additives has been shown to enable direct *in situ* preparation of porous monoliths. This type of sol–gel process has been utilized for successful preparation of titania- [19,20] and zirconia-based [15,18] monoliths and continues to be further explored.

Another method for direct preparation of porous monolithic structures employs metal salts. Progress in this area can be attributed to the initial work by Gash et al. [27–29]. The addition of suitable porogenic reagents to the reaction mixture containing a metal salt, a gelation reagent, and a solvent enhanced the sol–gel

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preparation of highly porous monoliths comprising hafnia [22,23], zirconia [22] and alumina [24,25].

In 2005, Baumann et al. [26] reported the preparation of alumina-based monoliths from hydrated aluminum nitrate and chloride precursors, each producing structures with significantly different properties. Porous alumina monoliths with well-defined macropores and mesoporous skeletons were reported by Tokudome et al. [24]. Two years later the same group published detailed structural characterization of the alumina aerogels and xerogels formed from hydrated aluminum chloride in the presence of a gelation reagent, propylene oxide, and a porogenic reagent, poly(ethylene oxide) [25].

This communication describes sol–gel preparation of porous alumina monoliths inside of fused silica capillaries that are suitable as the stationary phase for liquid chromatography. To the best of our knowledge, there is no report in the literature describing the application of porous alumina monoliths in capillary liquid chromatography. For the preparation of monolithic columns, we utilized two different salts, aluminum nitrate nonahydrate and aluminum chloride hexahydrate, the gelation reagent propylene oxide, and a mixture of porogen and a solvent, which also served the role of co-porogen. These columns were then used for the separation of a mixture of adenosine phosphates in order to demonstrate chromatographic use of the new material.

## 2. Experimental

### 2.1. *In situ* preparation of porous alumina monoliths in capillary

Prior to the preparation of alumina monoliths, a polyimide-coated fused silica capillary (100  $\mu\text{m}$  i.d., Polymicro Technologies, Phoenix, AZ) was rinsed with 1 mol/L sodium hydroxide, plugged with rubber septum and placed in an oven for 3 h at 120  $^{\circ}\text{C}$ . The capillary was then rinsed with water and acetone for 1 h followed by drying using a stream of nitrogen for 10 min and in the oven at 120  $^{\circ}\text{C}$  overnight.

Two types of alumina based monoliths, denoted as Al–N or Al–C, were prepared. Al–N was prepared by dissolving 1.086 g of  $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  in 300  $\mu\text{L}$  of acetonitrile, and 340  $\mu\text{L}$  of N-methylformamide at room temperature. Upon complete dissolution of the solid, 852  $\mu\text{L}$  of propylene oxide was added and the mixture was further stirred in ice until the solution was completely clear.

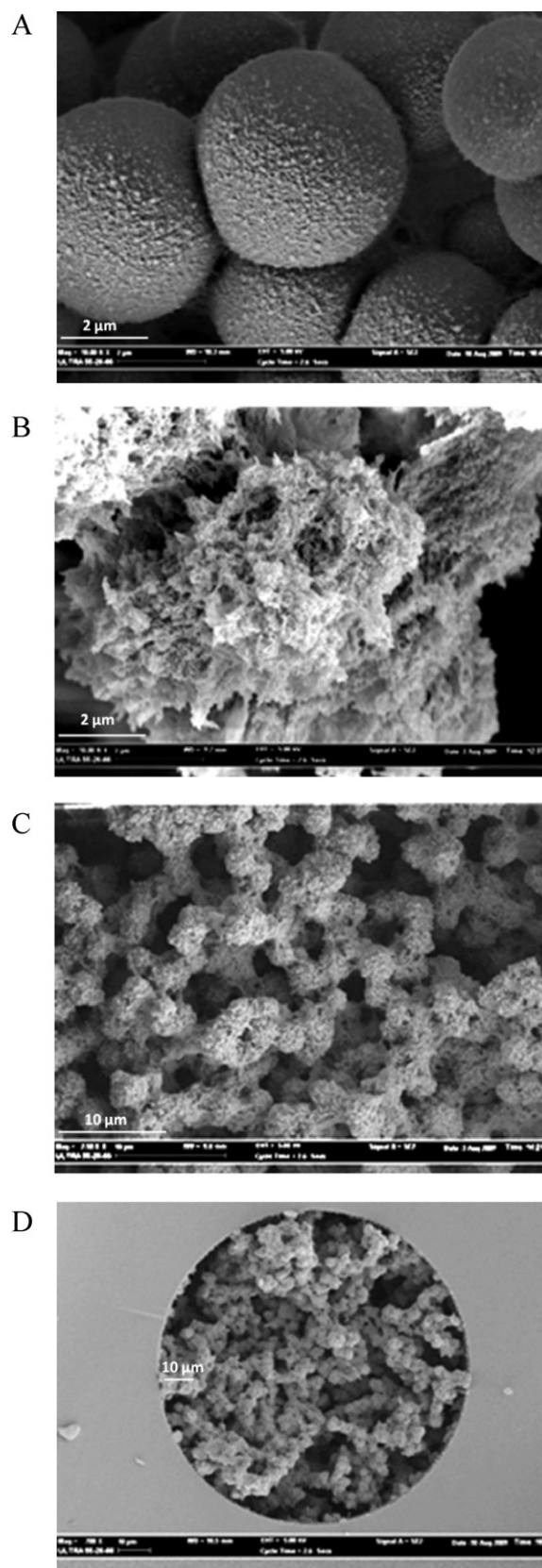
Al–C was prepared by dissolving 0.700 g of  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  in 300  $\mu\text{L}$  water/ethanol (1:1) mixture and 480  $\mu\text{L}$  of N,N-dimethylformamide (DMF) at room temperature. After a complete dissolution, 834  $\mu\text{L}$  of propylene oxide was added and the mixture was stirred in ice until the solution was completely clear.

In both procedures, the resulting homogeneous solution was charged into the pretreated fused silica capillary by hand-held syringe. Both, syringe and capillary were kept in ice prior to filling with the solution. If multiple capillaries were charged using the same solution, a new cold syringe was used each time. The sealed capillary was kept at 40  $^{\circ}\text{C}$  in a water bath for 2 days for aging. Then the precursor monolith was washed with ethanol at a flow rate of 1  $\mu\text{L}/\text{min}$  for 1 h unsealed dried in an oven at 40  $^{\circ}\text{C}$  for 1 day. Finally, the temperature was ramped to 120  $^{\circ}\text{C}$  at a rate of 0.5  $^{\circ}\text{C}/\text{min}$  and then held constant for 3 h.

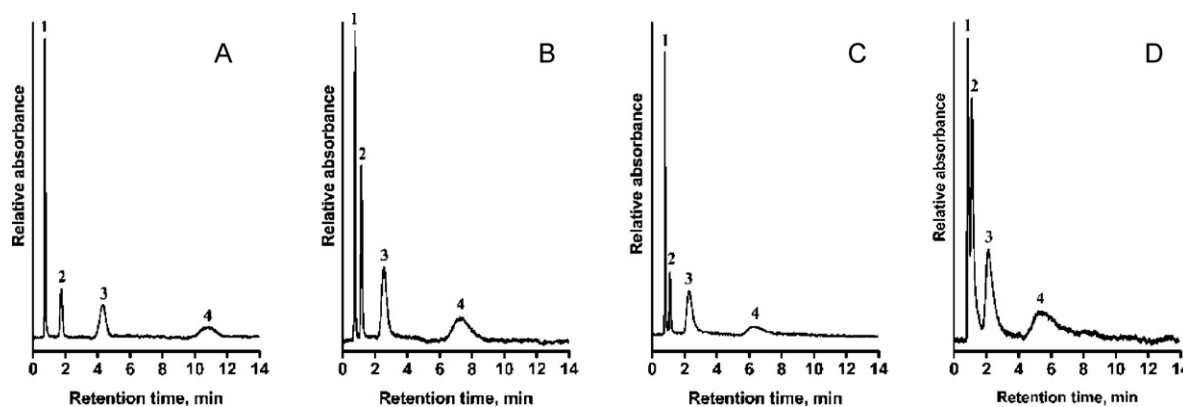
## 3. Results and discussion

### 3.1. Preparation of alumina monolith

During the sol–gel process, an aluminum oxide network is obtained through polymerization reaction of a metal salt precursor



**Fig. 1.** SEM images of alumina prepared from  $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  (A) and  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  (B–D).



**Fig. 2.** Separation of phosphorylated adenosine standards on the surface of Al-C at varying percentage of Na-P<sub>i</sub> buffer (A) 30%, (B) 40%, (C) 50%, and (D) 60%. Conditions: monolithic column 15 cm × 100 μm i.d.; mobile phase A: 50 mmol/L sodium phosphate (Na-P<sub>i</sub>) buffer pH 6.5; mobile phase B: acetonitrile; flow rate 1.5 μL/min; isocratic elution; UV detection at 254 nm. Peaks: adenosine (1); adenosine monophosphate (2); adenosine diphosphate (3); and adenosine triphosphate (4).

in the presence of gelation reagent. Thus, we first optimized reaction conditions to obtain porous alumina-based monoliths suitable as separation media for liquid chromatography. We examined the effects of variations in reaction parameters including type and concentration of aluminum salt precursor, gelation reagent, porogen, and solvent as well as temperature and duration of the aging and drying step.

SEM images in Fig. 1 show significant structural differences between capillaries prepared from hydrated aluminum nitrate (Al-N) or from chloride (Al-C). Formation of globular particle aggregates is observed when Al-N is used. It should be noted that the structure collapses upon heating to 120 °C and that these materials are soft and, due to the accompanying shrinkage, are most often detached from the capillary wall. In contrast, Al-C monoliths consist of well-defined interconnected skeletons and throughpores. Capillaries remain completely filled with the monolith that does not change its size while the temperature ramp is applied. The anions clearly play a different role in the process of the alumina network formation. According to Livage et al. [30] who studied chemistry of the sol-gel processes of transition metal oxides, chloride is a “non-complexing” anion. As such, it does not play a role during the hydrolysis and condensation steps. In contrast, the nitrate anion can coordinate to the aluminum cation and displace the terminal water molecules in the hydrated polymer chain. In addition, both anions modify the aggregation process to different degrees both through changes in the ionic strength of the solution and in the double layer composition.

The preparation of sol-gel from metal salts requires the presence of a gelation promoter, typically an epoxide. It was previously shown that homogeneous gelation in the presence of propylene oxide affords a monolithic gel [27–29]. This reagent is a proton scavenger, which undergoes an irreversible ring opening reaction in presence of a nucleophile. As a result, the slow rise in pH of the solution drives the hydrolysis and condensation of hydrated alumina species towards a monolithic gel formation. Phase separation that is critical for the preparation of monoliths with well defined throughpores is controlled by the amount of porogenic reagent N,N-dimethylformamide (DMF) or N-methylformamide (NMF). While both porogens afford the formation of white opaque monoliths, the monolithic structure cracked more frequently in the presence of N-methylformamide.

The effects of solvents with a wide range of polarities on the preparation of porous Al-N monoliths were studied as well. The solvent has a dual function: (i) it allows for aluminum salts to dissolve and (ii) it affects the reactivity of the nucleophile and thus the course of phase separation. A jelly-like structure was formed in the presence of water. Transparent and translucent

monoliths were obtained using formamide, N-methylformamide, N,N-dimethylformamide, methanol, and ethanol. However, phase separation was not supported using these solvents to the same degree as with 2-propanol and acetonitrile in which the formation of white opaque monoliths was observed. Heating the monoliths prepared in vials with 2-propanol lead to change in its color from white to yellow. In contrast, monoliths prepared in acetonitrile remained white after heating under the same conditions. This indicates that acetonitrile, a polar aprotic solvent, enhances the phase separation necessary for throughpore formation and the stability of the alumina skeleton.

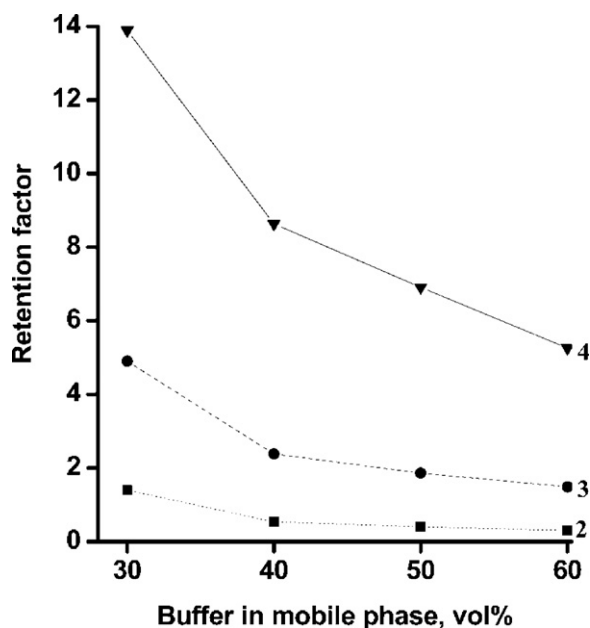
In addition to composition of the reaction mixture, the temperature and duration of the aging and drying steps also play an important role. We found that both room temperature and 40 °C were the most suitable choices; however, a longer aging time was required at room temperature. As a result, two days long aging at a temperature of 40 °C was optimal to afford formation of stable white opaque monoliths.

Prior to drying, monolithic capillaries were rinsed with ethanol in order to remove unreacted precursors and reaction byproducts [31]. This treatment is used in lieu of high temperature calcinations that cannot be carried out with polyimide coated capillaries. Subsequent one-day drying at 40 °C followed by slow increase of temperature to 120 °C was used to achieve solvent evaporation and gel drying.

### 3.2. Back pressure and permeability

A chromatographic system was used to determine pressure stability and permeability of prepared monoliths. Alumina monoliths obtained from various salt precursors exhibit a linear increase in the back pressure with an increase in the flow rate of acetonitrile. We noted that Al-C withstands pressures of up to 28.0 MPa, while Al-N collapses at a flow rate of 12 μL/min and a pressure of 6.7 MPa. The difference in back pressure for various capillaries is attributed to different morphology and mechanical stability.

The permeability of our columns relates to morphology and porosity and has a range of  $4.0 \times 10^{-10}$  to  $1.2 \times 10^{-9}$  cm<sup>2</sup>. Al-N columns exhibit higher permeability, which is desired since it allows higher flow rates and faster analyses. However, the limited pressure resistance and thermal stability restrict their use in liquid chromatography. Although Al-C columns feature lower permeability compared to Al-N, their better pressure and thermal stability make them more suitable for the desired application. Unfortunately, the small quantities of material in the capillary prevent direct measurement of porous properties.



**Fig. 3.** Changes of retention factor ( $k$ ) of adenosine monophosphate (2); adenosine diphosphate (3); adenosine triphosphate (4) at varying percentage of Na-P<sub>i</sub> buffer. Retention factors were calculated from retention times acquired in chromatograms shown in Fig. 2.

### 3.3. Separation of organic phosphates

Chromatographic properties of Al-C were assessed using a mixture of adenosine phosphates separated under isocratic conditions in a mobile phase composed of 50 mmol/L aqueous sodium phosphate (Na-P<sub>i</sub>) buffer pH 6.5 and acetonitrile at a flow rate of 1.5  $\mu$ L/min. Fig. 2 shows separations of adenosine and its monophosphate, diphosphate, and triphosphate derivatives using a mobile phase with an increasing percentage of Na-P<sub>i</sub> buffer.

Adenosine, which does not contain phosphate functionalities, is not retained in the column and elutes first. Its phosphorylated counterparts are then eluted in order of the increasing number of phosphate functionalities with adenosine triphosphate being retained most. The retention decreases with increasing percentage of buffer in the mobile phase (Fig. 3).

## 4. Conclusion

The optimized *in situ* sol-gel technique affords alumina-based monolithic columns featuring interconnected skeletons interspersed with throughpores that are stable at higher pressures and temperatures. These monolithic columns enable separation of phosphorylated adenosines under isocratic conditions. This ability of the alumina monolith can be ultimately applied towards assessing ATP activity of proteins and in proteomic studies concerned with phosphorylation of proteins. Current column efficiency found for the ADP is about 6200 plates/m. This is less than observed for the other metal oxide counterparts used for similar application [20]. However, our results indicate that further optimization of parameters of sol-gel preparation and optimization of separation conditions is likely to improve chromatographic performance. We

believe that monoliths with a higher proportion of smaller throughpores, adjusted by varying the amount of porogenic reagent, will increase the efficiency but may also lead to a decrease in column permeability.

## Acknowledgments

Experimental work carried out at the Molecular Foundry, Lawrence Berkeley National Laboratory and F.S. were supported by the Office of Science, Office of Basic Energy Sciences of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231. E.R. and Z.Z. were supported by the Department of Energy (DOE)/National Science Foundation (NSF) Faculty and Student Team (FaST) Program. In addition E.R. was supported by the NIH-NIGMS MARC U\*STAR grant T34 GM008021. Jana Krenkova from the Molecular Foundry is kindly acknowledged for her valuable suggestions helping implement this project.

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