

## ABSTRACT

Silica monolith column having mixed-mode i.e., hydrophobic (C<sub>18</sub>) and anion exchange interaction was successfully synthesized by on-column modification of the hybrid tetramethoxysilanes (TMOS) and methyltrimethoxysilanes (MTMS) using octadecyldimethyl-(*N,N*-diethylamino)silane (ODS) and ion exchange monomer of *N*-[3-(dimethylamino)propyl]acrylamide (DMPAA-Q) methyl chloride-quaternary salt, for the surface modification. The preparation of the ODS/DMPAA-Q silica monolith column was carried out in a fused silica capillary column of 75 µm internal diameter. At each modification stage, the ODS/DMPAA-Q silica monolith column was chromatographically characterized with micro liquid chromatography (µLC) and produced separation efficiencies of up to 43000 plates/min at the optimum velocity using the test mixture of alkylbenzenes, and 41800 plates/min for selected nucleotides. The ODS/DMPAA-Q silica monolith column was also characterized physically with scanning electron microscope (SEM), and showed to have an average through-pore size and skeleton size of 2.2 µm and 2.0 µm, respectively. The ODS/DMPAA-Q silica monolith column was then characterized in capillary electrochromatography (CEC) for the separation of inorganic and organic ions using a 50 mM phosphate pH 6.9 background electrolyte. The inorganic anions and organic ions were separated within 10 mins with column efficiency up to 114900 plates/min. The results suggest that modifying a silica monolith capillary column through in-situ polymerization of a monomer carrying a functional group can yield high efficiency columns for the ion-exchange-mode separation as well as for reversed-phased separations. The analysis of the porous silica monolith columns with CEC yields higher performance than CZE analysis for the separation of anions.

## ABSTRAK

Turus silika monolit yang mempunyai mod saling tindakan campuran iaitu hidrofobik ( $C_{18}$ ) dan penukar anion telah disintesis dengan jayanya melalui pengubahsuaian langsung ke atas permukaan silika di dalam turus hibrid *tetrametoksisilana* (TMOS) dan *metiltrimetoksisilana* (MTMS) menggunakan *oktadekilmetil-(N-N-dietilamino)silana* (ODS) dan monomer penukar ion iaitu garam metil klorida kuartenar *N*-3[-(dimetilamino)propil]akrilamida (DMPAA-Q). Penyediaan turus silika monolit dilakukan di dalam turus rerambut silika terlakur 75  $\mu\text{m}$  diameter dalaman. Di setiap peringkat pengubahsuaian, turus silika monolit ODS/DMPAA-Q dicirikan secara kromatografi dengan kromatografi cecair mikro, ( $\mu\text{LC}$ ) dan menghasilkan kecekapan yang tinggi sehingga 43000 plat/min pada kelajuan optimum menggunakan campuran uji alkilbenzena, dan 41800 plat/min untuk nukleotida terpilih. Turus silika monolit ODS/DMPAA-Q juga dicirikan secara fizikal dengan mikroskop pengimbas elektron (SEM), dan menunjukkan purata saiz lubang terus dan saiz partikel masing-masing adalah 2.2  $\mu\text{m}$  dan 2.0  $\mu\text{m}$ . Turus silika monolit ODS/DMPAA-Q kemudiannya dicirikan secara kapilari elektrokromatografi, (CEC) untuk pemisahan ion tak organik dan organik menggunakan 50 mM fosfat pH 6.9 sebagai elektrolit latarbelakang. Ion tak organik dan organik telah dipisahkan dalam masa 10 min dengan kecekapan turus sehingga 114900 plat/min. Keputusan ini menunjukkan pengubahsuaian turus silika monolit secara pempolimeran *in-situ* dengan monomer pembawa kumpulan berfungsi boleh menghasilkan turus berprestasi tinggi bagi pemisahan dalam mod penukar ion dan juga pemisahan fasa terbalik. Analisis dengan teknik CEC juga menghasilkan prestasi yang lebih tinggi berbanding analisis CZE bagi pemisahan anion.

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## CHAPTER 1

### INTRODUCTION

#### 1.1 General Introduction

Being the youngest member in the family of separation technique, capillary electrochromatography (CEC), is a micro column separation technique that combines the features of both capillary zone electrophoresis (CZE) and micro liquid chromatography ( $\mu$ LC) (Bartle and Mayers, 2001, Wu *et al.*, 2008). In CEC, the electroosmotic flow (EOF) generated on the surface of the stationary phase, is used to drive the mobile phase instead of hydrodynamic flow. Due to the plug-flow profile of the EOF, CEC exhibits separation of higher efficiency than in the pressure-driven  $\mu$ LC.

Separations of ionic solutes in CEC are commonly achieved with ion-exchange column packed with particles (Gu *et al.*, 2006; Norton and Shamsi, 2008, Tanaka and Kobayashi, 2003). However, there are several limitations that need to be solved on packed-CEC. One of the limitations of the conventional packed column is the necessity to fabricate frits, which is required for retention of the packed particles within the column. In addition, packed column also have the tendency to form bubbles around the packing materials or at the frits. Such problems often results in an unstable baseline, non-reproducible migration time and even, current breakdown. Moreover, in packed-CEC, the packing procedure is often more difficult compared to the normal High Performance Liquid Chromatography (HPLC) column due to the narrow inner diameter of the capillary (Bartle and Mayers; 2001). It is also observed that the solute band in this type of chromatographic column is broadened due to the

presence of multiple flow paths of different length and velocity, and slow equilibration of the solute between the mobile phase and the stationary phase, especially in nm-sized pores of  $\mu\text{m}$ -sized particles (Guiochon, 2007, Tanaka and Kobayashi, 2003). As to obtain separation with high efficiency, the use of small sized particles helps to reduce the contribution of the above mentioned factors, therefore enables faster equilibration and narrower bands to be achievable (Breadmore *et al.*, 2001; Ding *et al.*, 2006; Fu *et al.*, 2004; Jaafar *et al.*, 2008, Klampfl *et al.*, 2000, Lin *et al.*, 2006; Minakuchi *et al.*, 1996; Motokawa *et al.*, 2002; Tanaka and Kobayashi, 2003; Tang *et al.*, 2000).

The introduction of monolith stationary phases in the field of column technology is due to the problematic issues associated with packed column. The major chromatographic features of these monolith columns are providing high permeability based on its large through-pores, as well as high efficient separation with large number of theoretical plates per unit pressure drop based on small-sized skeleton (Breadmore *et al.*, 2001; Ding *et al.*, 2006; Fu *et al.*, 2004; Jaafar *et al.*, 2008, Klampfl *et al.*, 2000, Lin *et al.*, 2006; Minakuchi *et al.*, 1996; Motokawa *et al.*, 2002; Pesek *et al.*, 2008; Roux *et al.*, 2008; Tanaka and Kobayashi, 2003; Tang *et al.*, 2000). Guiochon (2007) defined monolith column as made up of one piece of continuous and porous material that is sealed against the wall of the tube. Due to this characteristic, the mobile phase will tends to percolate through it, avoiding bypassing any significant length of the bed. Other chromatographic advantages offer by this monolith material also include low backpressure and fast analytes mass transfer. In the aspect of monolith column preparation, the simplified preparation procedures which can be directly performed in-situ within capillaries has brought the monolith material to be gradually fabricated for application in microscale chromatographic separation such as in the pressure-driven  $\mu\text{LC}$  (Jaafar *et al.*, 2008; Pesek *et al.*, 2008; Roux *et al.*, 2008; Wu *et al.*, 2008) and the electro-driven CEC (Breadmore *et al.*, 2001; Ding *et al.*, 2006; Fu *et al.*, 2004; Jaafar *et al.*, 2008; Klampfl *et al.*, 2000; Lin *et al.*, 2006; Wu *et al.*, 2008). Technically, the preparation of monolith columns in electro-driven microscale capillaries is similar to that in pressure-driven  $\mu\text{LC}$ , except for the consideration of the generation of EOF for driving the mobile phase, which requires the incorporation of charged moieties onto the surface of the monolith.

Monolith stationary phase can be divided into two main groups which are silica-based monolith (Breadmore *et al.*, 2001; Ding *et al.*, 2006; Fu *et al.*, 2004; Jaafar *et al.*, 2008, Klampfl *et al.*, 2000; Lin *et al.*, 2006; Minakuchi *et al.*, 1996; Motokawa *et al.*, 2002) and organic polymer-based monolith (Uysal *et al.*, 2009, Wang *et al.*, 2009). Organic polymer-based monoliths are often prepared via a one-step polymerization of an organic monomer in the presence of a cross-linker, initiator and porogen. The results can be classified as either rigid or soft monoliths. As for silica-based monolith, it can be prepared by a sol-gel process whereby, alkoxysilanes will undergo a hydrolysis and polycondensation reaction catalyzed by acetic acid in the presence of porogens. The macroporous structure of the silica monolith prepared by sol-gel process is controlled by the composition of the starting materials. Therefore the size of silica skeletons and through-pore can be varied independently. Silica-based monolith column usually provides greater mechanical stability compared to the organic polymer-based monolith as the material show less swelling in solvents. One drawback of silica is its degradation above pH 7.5, whereas polymer-based stationary phase can withstand higher pH values. However, to circumvent this problem, weak acid eluents can be used instead of highly-alkaline eluents.

The improvement of selectivity in CEC is powered by the utilization of various packing materials as its stationary phase. Besides commercially available capillaries, novel stationary phase are also synthesized to provide sites of required interaction. Not only to widen the range of selectivity, novel aspect of the material is their ability to promote strong and constant EOF. Since stationary phase is the heart of separation technique, improvement of column technology is foremost important for the continued growth in CEC.

Here, we prepared a mixed-mode silica monolith column synthesized by modifying the hybrid silica monolith with C<sub>18</sub> (ODS) and quaternary ammonium functional group (DMPAA-Q), producing a mixed-mode of reversed-phase (RP) and a strong anion exchange stationary (SAX) phase for CEC. As a mixed-mode type of column, two different interactions of analytes towards the stationary phase is offered, thus enabling hydrophobic and positively charge compounds to be



separated. Physical characterization of the column exhibits skeleton and through-pore size within the range of that reported by Nakanishi *et al.* (1996), and it is expected that this column will produce good or similar performance in terms of its separation efficiency compared to other silica monolith column reported previously. A comparison study of the anions separation was also carried out on the CZE mode with a fused silica capillary.

## 1.2 Problem Statement

Reversed phase column with ODS functionality is widely applied as the stationary phase in chromatography due to its proven capability of separating neutral and hydrophobic analytes. Recently, strong anion exchange (SAX) column of DMAPAA-Q moiety has been proven successful in separating nucleotides and inorganic ions (Jaafar *et al.*, 2008). Incorporating the SAX functionality to an ODS column could afford a dual separation mode which can be applied to a wider range of analytes of organic species having hydrophobic properties; anion species, organic and inorganic; and hydrophilic substances, particularly amines. Better column performance could be expected from this mixed-mode column due to the additional interaction yield from both the moieties.

## 1.3 Significance of Study

In obtaining high efficient separation, silica monolith material is an ideal chromatographic separation medium to be applied in CEC, as the use of monolith helps to reduce the contribution of the band-broadening factors, therefore enables faster equilibration and narrower bands to be achievable. It is an added value in this research as not only physical characterization is carried out at the final stage, chromatographic characterization of the ODS/DMAPAA-Q silica monolith column at each modification stage is also conducted using  $\mu$ LC at certain chromatographic conditions. Analytes separation on normal CZE is also conducted as to distinguish

the separation influences on CEC that generate higher efficiency compared to the conventional CZE method.

#### **1.4 Objectives of Research**

The objectives of this research are to:

- (i) synthesize a silica monolith column having mixed-mode interaction of reversed-phased and ion-exchange using ODS and DMAPAA-Q monomer.
- (ii) conduct a chromatographic evaluation using  $\mu$ LC at each modification stage of silica monolith column. Those stages consist of (i) MAS-modified stage, (ii) ODS modified stage and (iii) ODS/DMAPAA-Q modified stage.
- (iii) conduct a physical characterization on the ODS/DMAPAA-Q silica monolith capillary using Scanning Electron Microscope (SEM) with the aid of Image Pro-Plus Analyzer.
- (iv) evaluate the performance of the ODS/DMAPAA-Q silica monolith column as an electrochromatographic separation medium in CEC using selected anionic compounds.
- (v) compare the efficiency and separation mode between the normal CZE and CEC for the separation of selected inorganic anions and ionic compounds.

#### **1.5 Scope of Research**

This research is carried out to explore the capability of mixed-mode monolithic silica as an electrochromatographic separation medium on selected anionic compounds (bromide, bromated, iodate, nitrate and benzoic acid) with CEC.

The ODS/DMAPAA-Q silica monolith is synthesized with the collaboration of Tanaka's Laboratory, Kyoto Institute of Technology, Japan.

The ODS/DMAPAA-Q silica monolith prepared in the fused-silica capillary of 75  $\mu\text{m}$  I.D. from the mixture of TMOS and MTMS first evaluated under  $\mu\text{LC}$  mode with specific chromatographic condition proposed by Motokawa *et al.* (2002). Evaluation based on column separation efficiency, are at the unmodified stage, modified ODS stage and lastly at the modification stage with ODS/DMAPAA-Q. The synthesized capillary is then tested for the separation of some common nucleotides. Since the mixed-mode monolithic silica is obtained in the form of capillary rod, physical characterization is limited to using SEM. The morphology study is conducted with the aid of Image Pro-Plus analyzer under cross-sectional view obtained from SEM.

The ODS/DMAPAA-Q silica monolith capillary with the mixed-mode functionalities is then applied as an electrochromatographic separation medium in CEC. The column is tested for the separation of selected anionic compounds and its performance is evaluated based on the calculation of theoretical plates,  $N$  and plate height,  $H$ . In this part of research, an optimum separation conditions are applied, adopted from the previous study (Jaafar *et al.*, 2008). Lastly, a comparison study of the ion analysis with the normal CZE mode is conducted and results obtained are discussed based on its separation elution and efficiency.