CHAPTER I

INTRODUCTION

1.1 Statement of purpose

The high performance liquid chromatography (HPLC) had become an interesting technique in both qualitative and quantitative analysis. The efficiency of HPLC column can be expressed by the Van Deemter equation (Equation 1.1) where A, B and C terms describe the band broadening due to eddy diffusion, longitudinal diffusion and resistances to mass-transfer, respectively. The expanded equation related to column parameters can be written as Equation 1.2 [3] where C_e, C_d, C_{sm}, C_m: constant plate-height coefficients, d_p: diameter of particle, D_m: the diffusion coefficient of the solute in mobile phase, u: linear velocity.

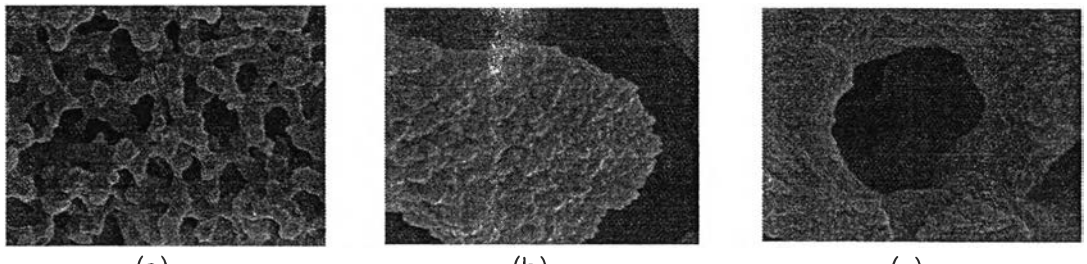
$$H = A + \frac{B}{u} + Cu \tag{1.1}$$

$$H = \frac{1}{[(1/C_e d_p)+(D_m/C_m d_p^2 u)]} + \frac{C_d D_m}{u} + \frac{C_{sm} d_p^2 u}{D_m} \tag{1.2}$$

The high efficiency can be obtained by decreasing plate height which is the decreasing of A, B and C terms. The decreasing of A and C terms was observed as the column and particle diameter decreased. But the high pressure pump (5,000 bar or more) is required for the small particles in packed column which is so called ultrahigh pressure liquid chromatography (UHPLC) using particle size about 2 μm and lower [4]. Although the high efficiency can be obtained by LC miniaturization, it is still leaving a problem with high back pressure. Therefore, the monolithic columns have been developed to overcome this problem [5, 6].

A monolithic column is a one-piece structure with interconnected skeleton which leads to large flow-through pores and high column permeability. A monolithic

stationary phase has a small-sized skeleton which can reduce the diffusion path length. In general, there are 2 types of pores in a monolithic column, macropores and mesopores, as shown in Figure 1.1. Two types of monolithic columns can be distinguished that is polymer monolith and silica-based monolith. Silica-based monoliths are better than polymer monolith with respect to organic solvent resistance, high mechanical strength and heat stability. Silica-based monolith can be prepared by sol-gel process which comprised hydrolysis and polycondensation reaction [7].



(a) (b) (c)

Figure 1.1 SEM images of a porous structure of monolithic silica column (a), the mesoporous structure (b), the macroporous or flow-throughpores structure (c) [8].

Generally, the preparation of silica monolith in capillary columns used TMOS (tetramethoxysilane) or TEOS (tetraethoxysilane) as a liquid precursor [9, 10]. Further development reported the addition of MTMS (methyltrimethoxysilane) in TMOS precursor to reduce shrinkage and protect void formation between wall column and silica skeleton [11]. In the past, monolithic columns were prepared in a large column diameter (4.6-7 mm.) by cladding it with frit at the end [12, 13] which is the difficult step. The shrinkage of monolithic silica skeleton often occurred by the preparation of monolithic silica column in large column diameter compared with small column diameter. The synthetic process of monolithic silica column for HPLC was shown in Figure 1.2. The monolithic silica column can be prepared in a fused-silica capillary (50-100 μm) without cladding with frit, which lessened the shrinkage [14].

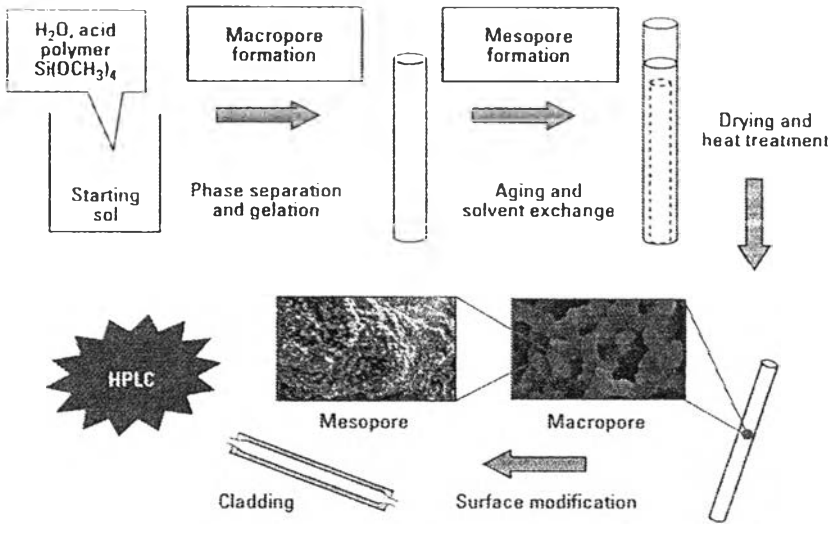


Figure 1.2 The synthetic process of silica monolithic column for HPLC [7].

The mesopores of silica monolith column are typically around 100-120 Å with surface area in the range of 140-300 m2/g, whereas the mesopores of organic polymer monolith column are around 2-50 nm with low surface area ~20-50 m2/g [15]. Therefore, the organic polymer monolith column suited a separation of large molecule such as protein [16, 17], nucleic acids [18]; where silica monolith column is suitable for fast separation of small molecules [19].

Because of these advantages of monolithic silica column above, many researchers reported the preparation of silica monolith and the modification method of bared silica monolith. There are many types of modified monolithic silica column such as normal phase [20, 21], reversed phase [22, 23], anion exchange [24, 25] and cation exchange [26, 27].

In the past, many reports modified a monolithic silica column for a reversed phase (RP) mechanism [2, 19, 28]. However, some of researches presented a mixed-mode mechanism which provides good separation efficiency for polar analytes and better stability and peak shape for basic analytes [25, 29]. For the best of our knowledge, there were three modification methods for a mixed-mode column that can be concluded. One of them was synthesized and modified a monolithic silica column at the same time [9]. Nevertheless the disadvantages of this procedure were the ratio of chemical modification reagent influenced the pore structure and continuous of skeleton of the column. The rest of the methods were a post-modification method where a monolithic silica column was first prepared and then

post-modified the silica surface with a designed group. Two methods of post-modification were reported which are one-step modification and two-step modification [10, 30]. The different modification method affected the structure and selectivity of each column. Hence, this work was to compare two monolithic silica columns which were modified with different post-modification methods for a mixed-mode mechanism.

1.2 Literature reviews

The advantages of a monolithic silica column are good solvent resistance and more strength than a monolithic polymer column because of an interconnected structure. There were many reports using silica monolith in chromatographic techniques such as capillary electrochromatography (CEC) and HPLC. The development was aimed to synthesize a bared silica monolith and modify method column for higher efficiency and resolving a tailing problem of basic analytes. Each modification method affected the selectivity of the column which is suitable for different samples.

In the past, many researches were published many modification methods of monolithic silica column for mixed-mode mechanism. In 2006 Ding et al. [8] prepared a reversed-phase with weak anion exchange monolithic capillary column for CEC by one step sol-gel procedure. The modification was accompanied with bared silica monolith synthesis by mixing of aminopropyltriethoxysilane (APTES), tetraethoxy-silane (TEOS) and octyltriethoxysilane (C_8-TEOS). The results showed that basic compounds can be separated on a mixed-mode monolithic column without peak tailing. As an alternative, Ye et al. [9] was prepared a bared monolithic silica capillary column and then modified the surface for CEC separation. The mixing of hexadecyltrimethoxysilane (HDTMS) and aminopropyltrimethoxysilane (APTMS) was introduced into a bared monolithic silica capillary column. The best column efficiency was obtained when the ratio of HDTMS to APTMS is 9 to 1. At this ratio, neutral and ionic compounds can be separated without peak tailing based on reversed phase mechanism due to the positively charged of amino groups which shielded the negatively charged of silanol groups. Moreover, the advantage of post-modification was mentioned that the ratios of modified reagent did not influence the skeleton and flow-through pores of a monolithic silica column.

Furthermore, many researches was studied a post-modification method with embedded polar groups. Ye et. al [30] developed C_{14} monolithic silica capillary column with embedded amine groups and used in CEC. The column was prepared by post-modification method with two-step modification. The first step was modified a bared monolithic silica column with glycidoxypropyltrimethoxysilane and the second step was modified the column with tetradecylamine. The results showed peak symmetry of neutral and basic compounds which refer to less silanol effect.

For HPLC technique, the particle packed column is the most development of stationary phase. O'Gara et al [31] modified base silica particles with various carbon atoms (C_8-C_{18}) and embedded with carbamate groups. The embedded polar group was prepared by the two-step modification method. The results showed that the number of carbon did not affect a peak symmetry. The tailing factors on carbamate-embedded column were less than that on C_{18} column. Moreover, the concentration of carbamate groups weakly affected the tailing factors.

In 2002, Layne [32] have compared the performance of 3 types of particles stationary phases in LC column as shown in Figure 1.3 (conventional C_{18}, a polar-embedded C_{18} and a polar-endcapped C_{18} phases). The results showed the decreasing of retention factor in hydrophobicity, and silanol activity at pH 2.5 on a polar-embedded C_{18} phase compared to a conventional C_{18} phase and a polar-endcapped C_{18} phase. However, at pH 7 conditions there was no significant different on silanol activity between a conventional C_{18} phase and a polar-embedded C_{18} phase. Therefore, a polar embedded (two-step method) and a polar endcapped (one-step method) stationary phase have become extensively interested in LC analysis. These phases can contribute the decreasing of silanol activity, improved peak shape of basic analytes and increased wettability of these phases for high aqueous mobile phase.

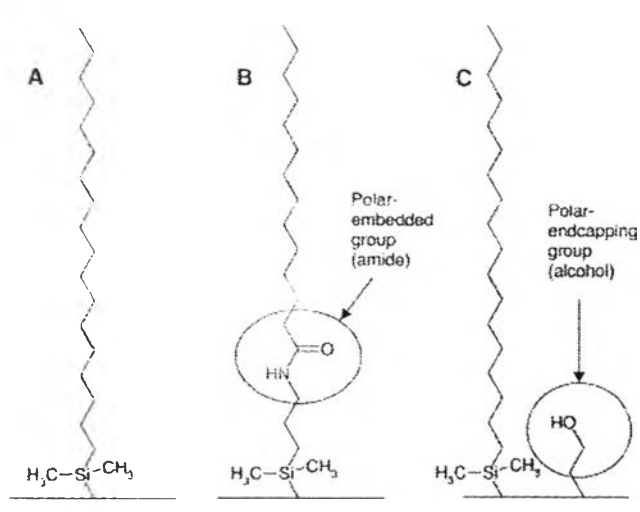


Figure 1.3 The structures for (a) a conventional C_{18} phase, (b) a polar-embedded phase, and (c) a polar-endcapped C_{18} phase [32]

1.3 Scopes of this research

1.3.1 Preparation of a bared monolithic silica capillary column by sol-gel process.

1.3.2 Functionalization of a bared monolithic silica capillary columns with (1) octadecyltrimethoxysilane (ODS) and aminopropyltrimethoxysilane (APTMS) (C_{18}-AP column) and (2) glycidoxypropyltrimethoxysilane (GPTMS) and tetradecylamine (C_{14}-amine embedded column)

1.3.3 Study chromatographic characterizations of the columns by Tanaka's procedure.

1.3.4 Separation of neutral, acidic and basic compounds on both columns by liquid chromatography.

1.4 The benefit of this research

Acquisition of the two types of monolithic silica capillary columns with different chemical modifier (C_{18}-AP phase and C_{14}-amine embedded phase) for reversed phase and weak anion exchange mechanisms on a mixed-mode liquid chromatography.