

## ACYLATION OF AMINOPROPYL-BONDED SILICA GEL FOR SPE

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### TÓM TẮT

#### **TỔNG HỢP PHA TĨNH THÔNG QUA PHẢN ỨNG ACYL HÓA AMINOPROPYL ĐÃ GẮN TRÊN SILICA GEL - ỨNG DỤNG TRONG CHIẾT PHA RẮN**

*Pha tĩnh pha đảo được tổng hợp theo phương pháp mới bằng cách acyl hóa các nhóm amino đã gắn trên bề mặt silica bằng tác chất stearoyl chloride. Các điều kiện phản ứng được tối ưu sao cho phần trăm cacbon của sản phẩm nằm trong khoảng trung bình so với các sản phẩm trên thị trường. Hàm lượng 3-aminopropylsilane và stearoyl chloride đều cân bằng với lượng dư để đạt được sản phẩm mong muốn. Với quy trình đơn giản như trên, pha tĩnh tạo ra có %C lên đến 14.2% và chi phí bằng hai phần ba so với cách tổng hợp truyền thống. Bên cạnh tính chất của pha đảo thông thường, việc 60% nhóm amino chưa phản ứng làm cho pha tĩnh có thể tương tác đa cơ chế. Sự khác biệt về tính chất lưu giữ của pha tĩnh tổng hợp trong nghiên cứu này được so sánh với pha tĩnh C18 thông thường. Kết quả cho thấy pha tĩnh tự tổng hợp lưu giữ tốt hơn các chất có tính acid, qua đó khả năng làm sạch mẫu với các chất phân tích này là tốt hơn.*

#### 1. INTRODUCTION

Solid phase extraction (SPE) is a well-established sample pretreatment technique because it demands less organic solvents and can remove interferences simultaneously. In SPE chemical structure of sorbent materials play a decisive role [1]. Currently, the most popular sorbent material is conventional reversed phase (CRP) on silica support. CRP is synthesized by directly bonding octadecylsilane onto

silica surface[2, 3]. However, expensive silylation reagents increase the cost of production. This disadvantage could be reduced by using amide-reverse stationary phase (amide-RP) since reagents for preparation are cheaper. Amide-RP normally is synthesized in a two-step process, (i) grafting 3-aminopropyl ligand onto silica surface by reaction with 3-aminopropylsilane and (ii) acylation of amino groups with stearoyl chloride (STC)

to graft C17 chains through polar amide bonds[4, 5]. While the first step is auto-catalyzed reaction, the second requires a base catalyst[6]. The resulted product remains free amino groups since the amide formation reaction gave only 20-50% in yield[4]. The dual ligand possession can lead to additional polar interactions in addition to the conventional reversed phase partition.

In the current study, amide-RP sorbent was synthesized and its retention properties were compared with three commercial CRPs.

## 2. EXPERIMENTAL

### 2.1. Marterials

Silica (particle diameter 40-60 $\mu$ m, mean pore diameter 60Å, specific surface area 500 m<sup>2</sup>/g), 3-aminopropyltrimethoxy silane (APTS) 95% and stearyl chloride (STC) 97% were purchased from Scharlau, Acros and Sigma-Aldrich, respectively.

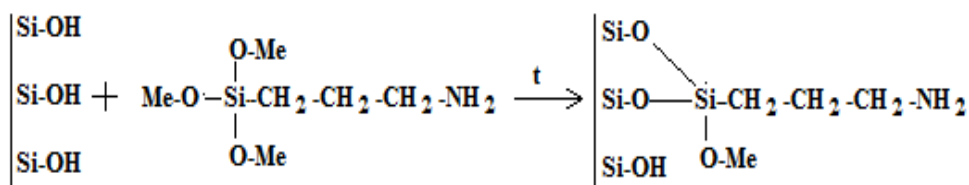


Figure 1. Reaction of silica and 3-aminopropyltrimethoxysilane

### 2.4. Preparation of amide-silica reversed phase (Amide-RP) and cartridge

AP-Si (2g) was suspended in toluene (20mL), then stearyl chloride (3.2 ml, 9.76 mmol) and imidazole (0.5 g, 7.35 mmol) were added to the mixture. The mixture

Imidazole, pyridine, formic acid were products of Merck, diethylamine, toluene, acetonitrile for HPLC were purchased from Labscan. Five analytes consist of caffeine, sulfadimethoxine, bromacil, warfarin, prednisone were obtained from Sigma-Aldrich.

### 2.3. Preparation of aminopropyl-bonded silica (AP-Si)

Silica gel was dried at 200 °C for 24 h. After cooling to room temperature, 2 g of silica were added to a flask, then 20 mL of toluene and 1mL of APTS were added. The mixture was heat to 100 °C under refluxed and paddle stirred condition for 4 h. The product was filtered and washed three times successively with 30 mL portions of toluene, methanol, deionized water, acetone and finally dried at 50 °C for 2 h.

was stirred with a paddle and refluxed at 100 °C for 3 h. The resulted product was filtered and washed three times successively with 30 mL portions toluene, petroleum ether, diethylether, acetone and finally dried at 50 °C for 2 h.

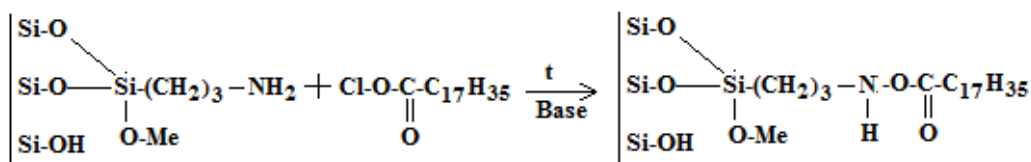


Figure 2. Reaction of AP-Si and stearyl chloride

Reaction conditions were optimized, namely reaction time (0.5-5 h), temperature (60-100 °C), identity of catalyst (diethylamine, triethylamine, pyridine, imidazole) and mole ratio of catalyst to nitrogen.

Cartridge for SPE were prepared by packing 500 mg of absorbent into a 6mL syringe, and the material was retained in SPE cartridges by polyethylene frits.

## 2.5 Sorbent material characterization

### 2.5.1. Elemental analysis of the resulted sorbent

To calculate the reaction yield, carbon and nitrogen content of product were determined.

- Nitrogen content was analyzed by Kjeldahl method[7]. Briefly, mixture of Na<sub>2</sub>SO<sub>4</sub>:CuSO<sub>4</sub> (9:1, w/w) (5g), H<sub>2</sub>SO<sub>4</sub> conc. (10 mL) and the sorbent (0.2 g) were heated at high temperature until the mixture color transferred from black to Cu<sup>2+</sup> blue. Resulted NH<sub>3</sub> was distilled and absorbed to 20mL of 0.5N H<sub>3</sub>BO<sub>3</sub> acid solution and titrated with H<sub>2</sub>SO<sub>4</sub> 0.02N

- Carbon content was analyzed by Alison wet-oxidizing method[7]. Briefly, mixture of K<sub>2</sub>CrO<sub>7</sub> (4 g), H<sub>2</sub>SO<sub>4</sub> (concd):H<sub>3</sub>PO<sub>4</sub> (concd) (3:2, v/v) (30mL) were used to oxidize the sorbent material (0.3 g) and produce CO<sub>2</sub>. The resulted CO<sub>2</sub> was absorbed to 20mL of 0.25N NaOH solution and titrated with HCl 0.2N.

### 2.5.2. Evaluation of retention

To evaluate retention properties of amide-RP, five analytes with different chemical properties were used (Table 1). The

mixtures of the five compounds (10µg/mL) in water were loaded onto the home-made amide-RP cartridges and other three commercial C18 cartridges (Agilent SampliQ C18, Strata C18-E and Isolute C18), the bounded analytes were then eluted with 5 mL mixtures of methanol and water. The collected eluents were analyzed by HPLC to calculate the recoveries.

*Table 1. Base-acid characteristics of five standards used for testing retention of amide-RP*

Compound	Class	Log P
<b>Caffeine</b>	Base	-0.13
<b>Sulfadimethoxin</b>	Acid	1.48
<b>Predneson</b>	Neutral	1.57
<b>Bromacil</b>	Base	2.1
<b>Warfarin</b>	Acid	3.42

### Chromatography conditions

HPLC separations were performed with Agilent 1100 system, UV detector, Ascentis™ C18 Supelco separation column (25cm × 4.6mm, 5µm). Detection wavelength was of 254 nm, temperature of 40°C. Aqueous 0.1% formic acid (phase A) and acetonitrile 0.1% formic acid (phase B) were used as mobile phase at 1 mL.min<sup>-1</sup>. The mobile phase gradient program was started with 15% of phase B, increased to 40% phase B for 14 min and to 100% for 22 min.

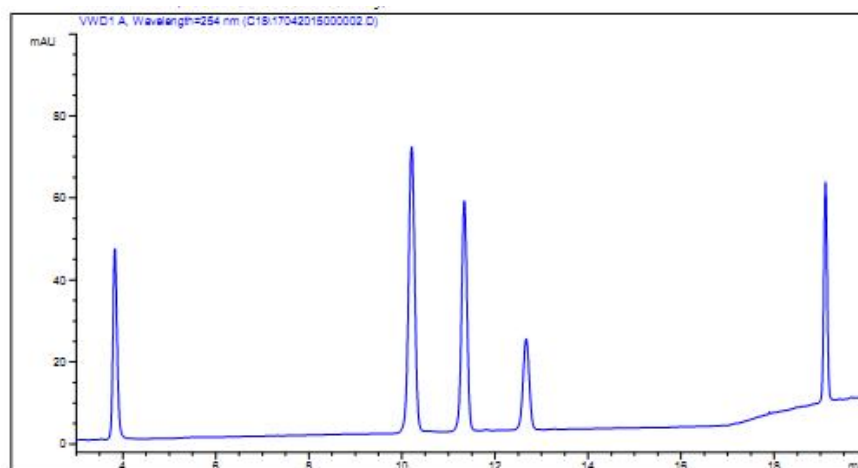


Figure 3. Chromatogram of tested standards, (1) Caffein, (2) Sulfadimethoxin, (3) Predneson, (4) Bromacil, and (5) Warfarin

### 3. RESULTS AND DISCUSSION

#### 3.1. Synthesis conditions for AP-Si

It is well-known that the reaction of silica and APTS is usually carried out at high temperature ( $\sim 100^\circ\text{C}$ ) in toluene, above 3 h and it is auto-catalyzed by the amino groups of the reagent [5, 6, 8]. In this work, no further investigation was conducted to optimize these reaction conditions. However, amount of 3-aminopropylsilane should be investigated for an expected % N of ca. 1.7 %. Reason for this is described in 3.2.. It was found that 1 mL APTS/2g silica was required for  $\sim 1.7\%$  of nitrogen content.

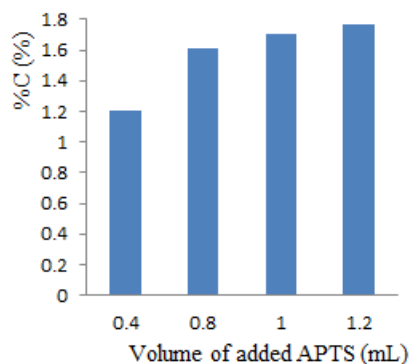


Figure 4. Effect of added volume of APTS on APTS-Si

#### 3.2. Synthesis conditions for amide-RP

Since commercial CRPs have from 10-18% in %C, we expected amide-RP possessed %C in a similar range. Many papers showed that acylation of amino groups bonded on silica surface gave the largest reaction yield of 20-50% (calculated from reacted nitrogen groups). N content of AP-Si of about 1.7% was chosen because if acylation reaction was optimized from 35-45% in yield, corresponding amide-RP would have %C in the expected range. All other reaction conditions such as catalyst, time and temperature were optimized to achieve desired products while mole ratio of STC to N was kept at 4.

##### 3.2.1. Effect of catalyst

Due to the formation of acid by-product, a base must be present in order for the acylation reaction to proceed. Figure 5 shows that a slight increase in %C ( $\sim 6\text{-}8\%$ ) relative to without catalyst was observed with diethylamine and pyridine. This was attributed for their weak base property and low boiling point. Only imidazole gave the highest %C (14.2%) because of its strong base property and high boiling point. 14.2% of C corresponds to reaction yield of 40%.

As a result, imidazole was selected for subsequent investigations.

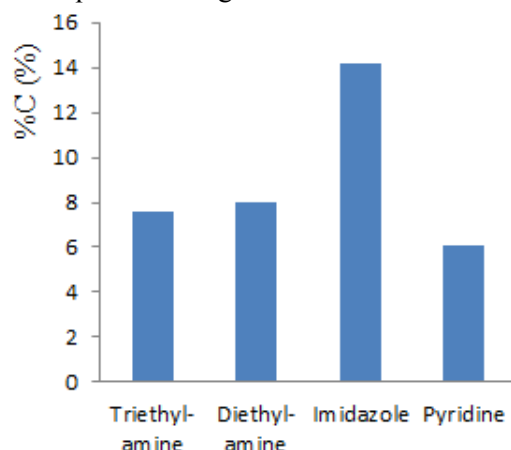


Figure 5. Effect of catalysts on amide formation reaction

Amount of imidazole was also an important factor and should be studied. Figure 6 showed that %C of product was largest when the mole ratio of imidazole to nitrogen was of 3.

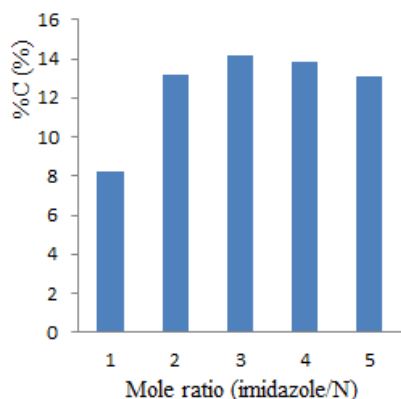


Figure 6. Effect of mole ratio of imidazole to N of AP-Si on acylation reaction

There were a slight decrease in %C with higher mole ratios, this was due to hydrolysis of the Si-O-Si linkage between silica surface and APTS. As a result, the mole ratio of 3 was maintained in subsequent experiments.

### 3.2.2. Effect of reaction temperature

The amide formation reaction from primary amine and chloride acid was first

described in 1983 by Carl Schotten and Eugen Baumann[9].

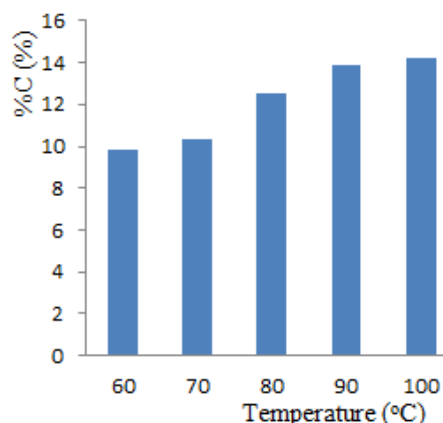


Figure 7. Effect of temperature on amide formation reaction

Most studies performed the reaction under refluxed condition [4, 5, 10, 11]. In this work a gradual increase in %C was seen as reaction temperature increased, and reached 14.2% at 100°C which was slightly lower than the boiling point of solvent (toluene, 110°C)(Fig.7). 100°C was consequently chosen for subsequent experiments.

### 3.2.3 Effect of reaction time

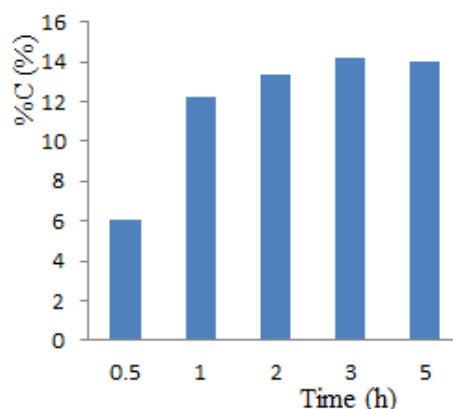


Figure 8. Effect of reaction time on amide formation reaction

As can be seen from Figure 8, the reaction yield almost reached the maximum with %C of 14.2. As a result, the reaction should be carried out for 3 hours.

### 3.3. Retention behavior

The five analytes with different acid base properties (Table 1) were used in testing the

retention behavior of the amide-RP sorbent in comparison to the commercial C18 sorbents

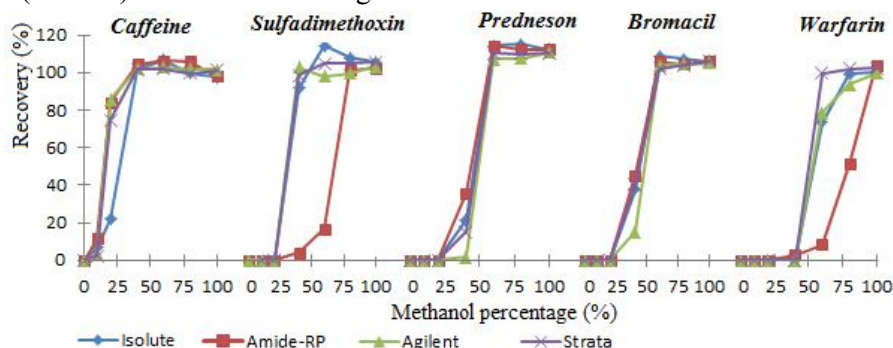


Figure 9. Elution profiles of tested analytes with amide-RP and three commercial RP sorbents

Elution profiles shows that the amide-RP had similar retention behavior as the other three commercial RP for neutral and base compounds such as caffeine, prednisone, bromacil while stronger retention was observed in case of acid compounds such as sulfadimethoxin, warfarin. Therefore, the ability of interference reduction of the amide-RP for acid analyte was better than that of conventional C18 phase. As for sulfadimethoxin, warfarin, eluents with up to 60% of methanol could be used to remove impurities but still the recoveries of more than 90%.

### 4. CONCLUSIONS

Amide embedded reverse phase was synthesized through a two-step procedure. The resulted amide-RP possessing carbon loading of 14.2% and with the cost of ca. two thirds of those for conventional RP. Since 60% of amino groups were still unreacted, the stationary phase had mixed-mode mechanism. Accordingly, beside the characteristic of RP the amide-RP had better affinity to acidic compounds.

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