

Synthesis of the reversed stationary phase for solid phase extraction using trimethoxyoctadecyl silane

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(Received on July 20th 2015, accepted on March 31th 2016)

ABSTRACT

Octadecyl grafting silica was synthesized using trimethoxyoctadecyl silane. Reaction conditions were optimized so that the carbon percentage of resulted material was similar to that of commercial products. Temperature and catalyst played very important roles in the reaction. The material was also undergone the end-capping process to reduce unreacted silanol

Key words: reversed stationary phase, octadecyl modified silica, alkoxyoctadecyl silane, solid phase extraction

groups. Final product owned 14.1 % of carbon content and 75 % of the unreacted silanols. Retention behavior of product was tested and compared to the same ones available on the market. Results showed that both resulted material and commercial had the same retention properties.

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 alkoxyoctadecyl silane or chlorooctadecyl silane onto silica surface[2-5]. While chlorosilane is very reactive, alkoxyoctadecyl silane is one of the least reactive reagents[3]. However, during the reaction with silica, chlorosilane produces HCl by-product which can damage the main product. For alkoxyoctadecyl silane, the by-product is alcohol which is harmless and easy to remove in distilled conditions. Besides, the less reactivity of alkoxyoctadecyl silane can be overcome by employing an appropriate catalyst [6].

In the current study, CRP sorbent was synthesized using alkoxyoctadecyl silane and its retention properties were compared with commercial CRPs.

MATERIAL AND METHODS

Materials

Silica (particle diameter 40-60 μm , mean pore diameter 60Å, specific surface area 500 m^2/g), were purchased from Scharlau. Trimethoxyoctadecyl silane (TMOS) 90 % and Chlorotrimethyl silane (97 %) was product of Sigma-Aldrich.

Imidazole, *p*-toluenesulfonic acid, 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.

Preparation of CRP

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, 0.5 mL of TMOS and 150 mg *p*-toluenesulfonic acid were added. The mixture was heated to 100 °C under reflux and paddle

stirred condition for 4 h. The product was filtered and washed three times successively with 30 mL portions of toluene, diethylether, methanol, acetone and finally dried at 50 °C for 2 h.

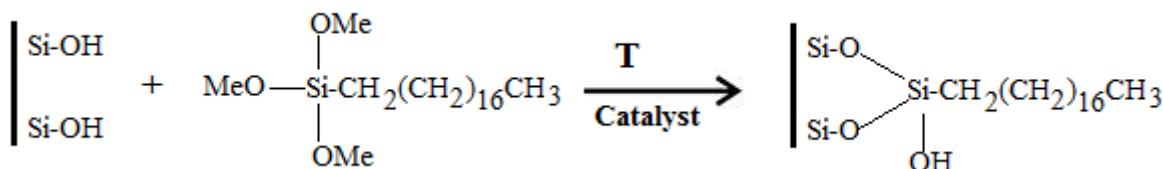


Fig. 1. Reaction of silica and trimethoxyoctadecyl silane

End-capping of CRP

To a 100 mL flask, add 1 g of CRP, 15 mL of toluene, 1 mL of chlorotrimethyl silane and 0.5 g of imidazole. The mixture was heated to 80 °C under reflux and paddle stirred condition for 4 h. The final product was filtered and washed three times successively with 30 mL portions of toluene, methanol, acetone and finally dried at 50 °C for 2 h.

Sorbent material characterizations

The carbon content was analyzed by Alision wet-oxidizing method [7]. Briefly, amixture of K₂CrO₇

(4 g), H₂SO₄ (concd):H₃PO₄ (concd) (3:2, v/v) (30 mL) were used to oxidize the sorbent material (0.3 g) at high temperature (approximately 200 °C) and produce CO₂. The resulted CO₂ was absorbed to 20 mL of 0.25 N NaOH solution. Blank sample was also performed by substituting silica for sorbent material. By titrating the NaOH solutions of blank and material samples with HCl 0.25 N and phenolphthalein as indicator, carbon content can be calculated as the following formula:

$$C (\%) = \frac{(V_{Bl} - V_s) \times C_{HCl} \times 12}{m (g) \times 10}$$

V_{Bl}: Volume of HCl solution to titrate absorption solution of blank sample (mL)

V_s: Volume of HCl solution to titrate absorption solution of sample (mL)

C_{HCl}: Concentration of HCl solution (M)

m: Weight of sample (g)

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

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

Evaluation of retention

To evaluate retention properties of resulted CRP, five analytes with different chemical properties were used (Table 1). The mixture of the five compounds (10 µg/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 mixture of methanol and water. The collected eluents were analyzed by HPLC to calculate the recoveries.

Chromatography conditions

HPLC separations were performed with Agilent 1100 system, UV detector, Ascentis™ C18 Supelco separation column (25cm × 4.6 mm, 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⁻¹. 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.

RESULTS AND DISCUSSION**Synthesis conditions for CRP***Effect of catalyst*

Catalyst is a very important element in silanization. Reaction of octadecyltrimethoxy silane and silica gave only 3.5 % in carbon content without catalyst. The reaction of surface silanols with alkoxy silanes can be catalyzed by either acids or bases [6]. As the mechanism showed in figure 2, before removing water to form the linkage to silica surfaces at high temperature, the alkoxy groups of alkoxy silanes are hydrolyzed to form intermediate silanols which are very reactive. Catalyst accelerates the hydrolysis (1) and condensation (2).

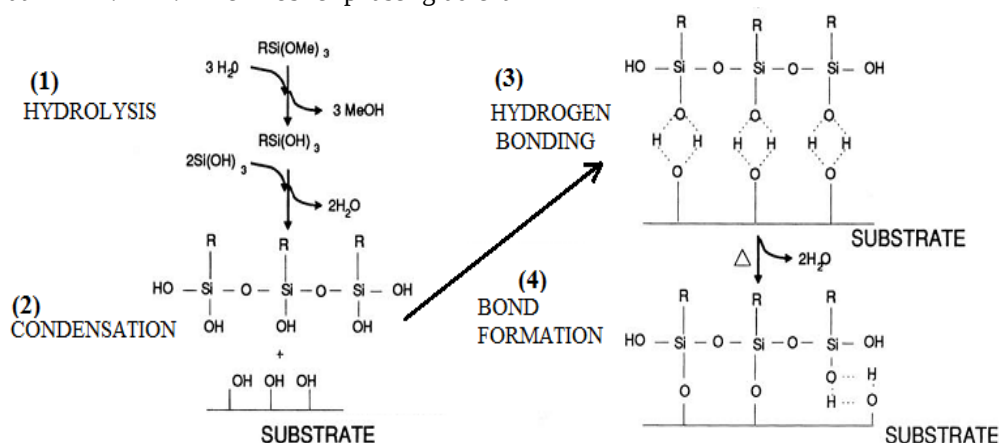
**Fig. 2.** Reaction mechanism of alkoxy silane and silanol.

Table 2. Properties of some organic acids and bases.

Catalyst	pK_b	pK_a	B_p ($^{\circ}C$)
Pyridine	8.7	-	115.2
Triethylamine	3.2	-	88.6
Diethylamine	3.1	-	55.5
Imidazole	7.0	-	256
<i>p</i> -toluene sulfonic acid	-	-2.8	140

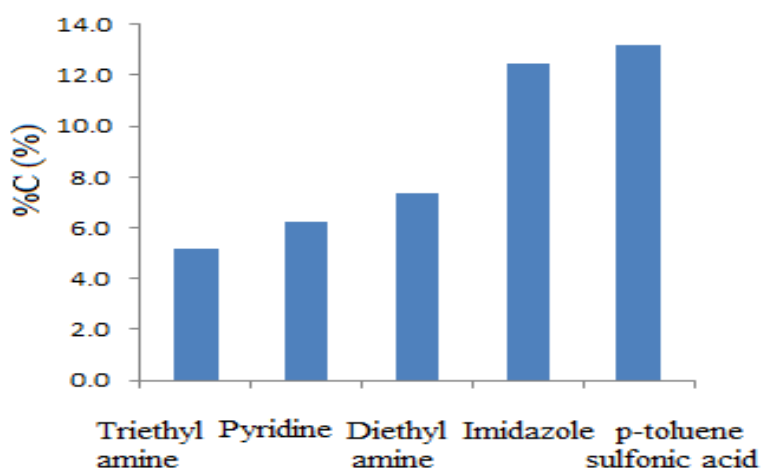
**Fig. 3.** Effect of catalyst on reaction of silica and trimethoxyoctadecyl silane

Fig 3 shows that bases as triethylamine, pyridine and diethylamine had weak effect on the silanization, and the carbon content of the obtained product was from 6 to 8 %. This could be due to their weak basic property and low boiling point (Table 2). Imidazole was a better catalyst with 12.5 % in carbon amount. However, the best was *p*-toluenesulfonic acid with

13.2% of carbon loading, corresponding to the reaction yield of 90 %. This was similar to results of the previous publication [6]. As a result, *p*-toluenesulfonic acid was selected for subsequent investigations.

Effect of amount of catalyst

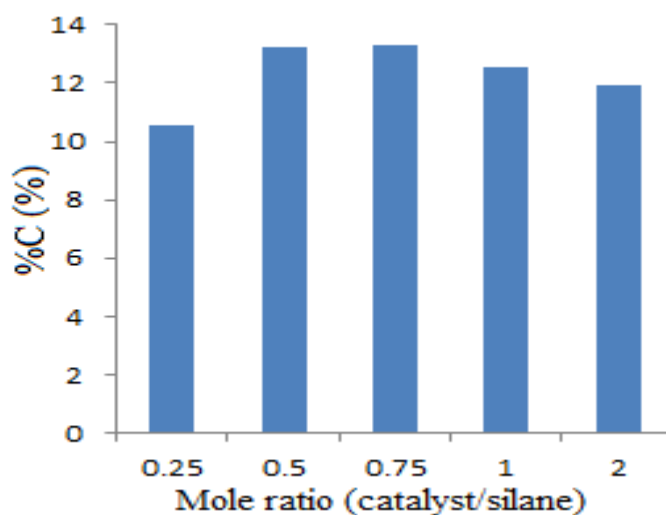


Fig. 4. Effect of catalyst amount on reaction of silica and trimethoxyoctadecyl silane

As can be seen from Fig. 4, good yield (90 %, corresponding to % C of 13.2 %) was obtained when the number of moles of *p*-toluenesulfonic acid was a half compared to that of silane. Consequently the mole ratio of 0.5 was selected for the following investigations.

As can be seen from step 4 of silanization mechanism (Fig. 2), the temperature is a very essential factor and need to be optimized. Besides, solvent also needs to be investigated because it affects the solubility of the reagent and the permeability of silica.

Effect of solvent and temperature

Table 3. Properties of some solvents

Solvent	Xylene	Toluene	1,2-dichlorobenzene	Benzene
B _p (°C)	138.5	110	180	80
Dipole moment (D)	0.07 – 0.64	0.39	1.80	0

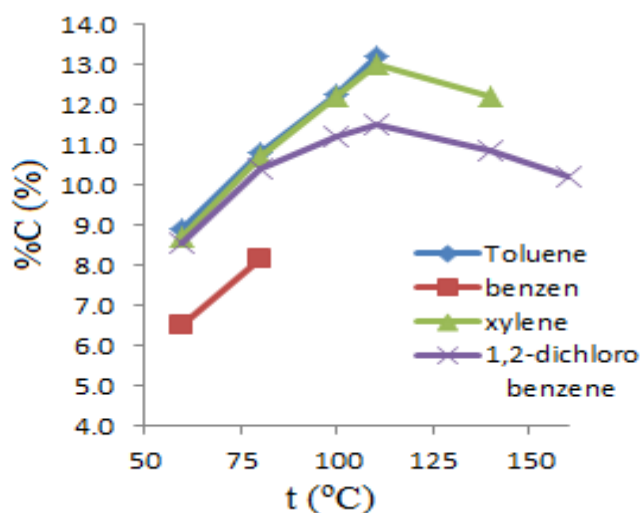


Fig. 5. Effect of temperature and solvent on reaction of silica and trimethoxyoctadecyl silane

Fig. 5 shows that the carbon percentage of CRP raises as the reaction temperature increases. However, solvents possessing too low or high polarity such as benzene or 1,2-dichlorobenzene gave low reaction yield. Toluene and xylene had the same trend at

temperature lower, than 110 °C, and highest carbon amount of product with these solvents reach up to 13.2 %. Since toluene is less toxic than xylene, it was consequently chosen for subsequent experiments.

Effect of reaction time

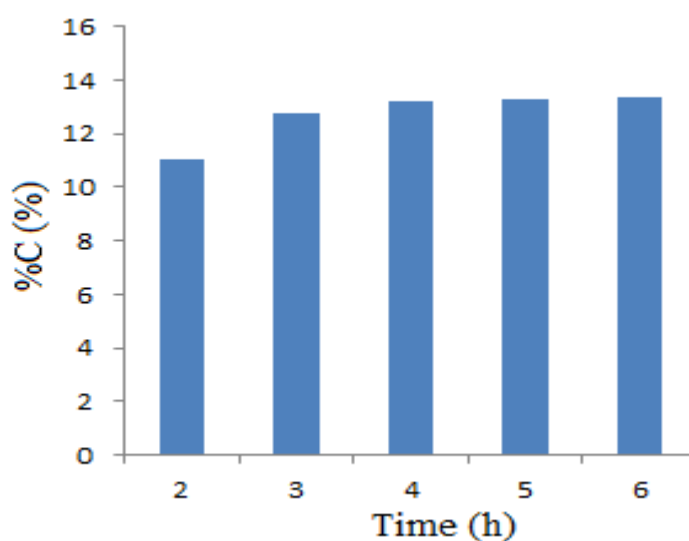


Fig. 6. Effect of time on reaction of silica and trimethoxyoctadecyl silane

Fig. 6 shows that the reaction of octadecyltrimethoxy silane and silica happened very fast at the first two hours and then gradually slow down. After 4 hours the carbon loading almost reached the maximum with of 13.2 %. As a result, the reaction should be carried out for 4 h.

End-capping

The surface coverage of silica with octadecyl silane could not have high density due to steric

hindrance. In an effort to reduce unreacted silanol groups, significantly smaller silanes were used, so-called end-capping process [8, 9]. In this current work, the end-capping process was conducted as in Patent EP1361915 B1 with chlorotrimethyl silane without further investigations. Table 4 shows that after the end-capping process completed, a slight increase of the carbon content of CRP (14.1) was observed.

Table 4. Properties of resulted material before and after end-capping process

CRP type	No end-capping	End-capping
Carbon percentage (%)	13.2	14.1
Unreacted silanol amount (%)	88.0	75.3

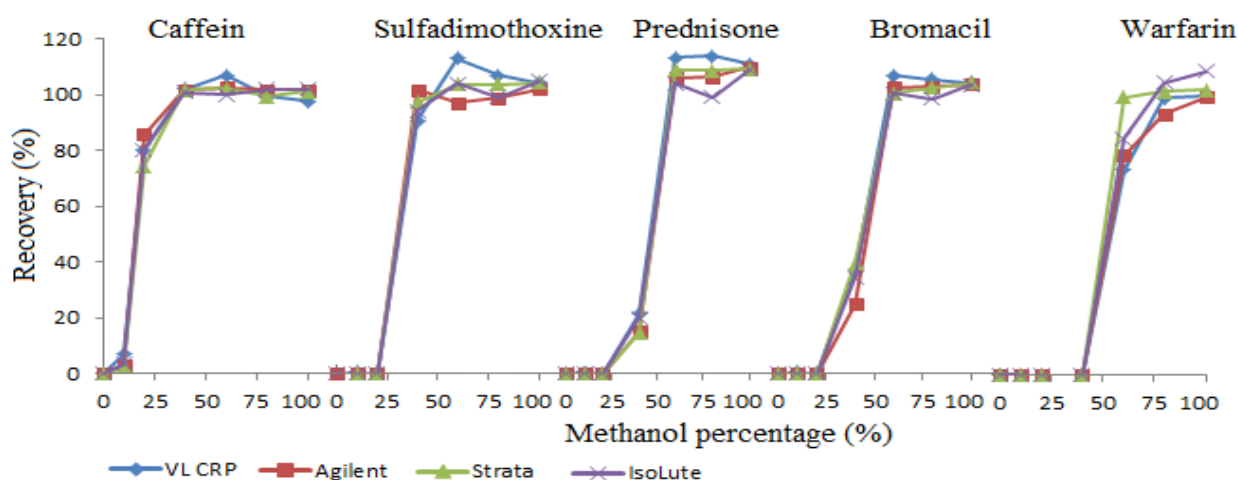


Fig. 7. Elution profiles of tested analytes with resulted material and three commercial CRPs sorbents.

Retention behavior

The five analytes with different acidic or basic properties (Table 1) were used in testing the retention behavior of the CRP sorbent in comparison to the commercial C18 sorbents.

Elution profiles show that resulted CRP had similar retention behavior as the three other commercial CRPs for all acid and base compounds. Therefore, the ability of purifying and concentrating analytes of resulted CRP were the same as the others.

CONCLUSION

Octadecyl modified silica was synthesized using trialkoxyoctadecyl silane reagent. The of reaction yield between silica and the silane reagent largely depended on the temperature and catalysts. Resulted material was end-capped and possessed 14.1 % of the carbon content. The retention behaving of the obtained product was similar to those of the same commercial products. Therefore, it was able to purify and concentrate analytes as much as other available product.

Tổng hợp vật liệu pha đảo dùng trong chiết pha rắn với tác chất trimethoxyoctadecyl silane

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TÓM TẮT

Vật liệu pha đảo trên nền silica được tổng hợp với tác chất trimethoxyoctadecyl silane. Các điều kiện phản ứng được tối ưu sao cho sản phẩm tạo ra có hàm lượng carbon đạt trong khoảng trung bình so với sản phẩm thương mại cùng loại. Kết quả cho thấy phản ứng giữa các nhóm silanol bề mặt và tác chất silane phụ thuộc rất nhiều vào nhiệt độ và xúc tác.

Từ khóa: chiết pha rắn, SPE pha đảo, silica, trialkoxyoctadecyl silane

TÀI LIỆU THAM KHẢO

- [1]. D. Luo, Q.W. Yu, H.R. Yin, Y.Q. Feng, Humic acid-bonded silica as a novel sorbent for solid-phase extraction of benzo [a] pyrene in edible oils, *Analytica Chimica Acta*, 588, 261-267 (2007).
- [2]. K.A. Lippa, L.C. Sander, R.D. Mountain, molecular dynamics simulations of alkylsilane stationary-phase order and disorder. 1. Effects of Surface Coverage and Bonding Chemistry, *Anal Chem*, 77, 7852-7861 (2005).
- [3]. J.N. Kinkel, K.K. Unger, Role of solvent and base in the silanization reaction of silicas for reversed-phase high-performance liquid chromatography, *Journal of Chromatography A*, 316, 193-200 (1984).
- [4]. M. Hetem, J. de Haan, H. Claessens, P. Mussche, C. Cramers, Effect of acid pretreatment of the silica substrate on the stability of octadecyl modified reversed phases, *Chromatographia*, 29, 473-481 (1990).
- [5]. B. Buszewski, L. Nondek, A. Jurášek, D. Berek, Preparation of silanized silica with high ligand density. The effect of silane structure, *Chromatographia*, 23 442-446 (1987).
- [6]. H. Engelhardt, P. Orth, Alkoxy silanes for the preparation of silica based stationary phases with bonded polar functional groups, *Journal of Liquid Chromatography*, 10, 1999-2022 (1987).
- [7]. A.L.P.R.H.M.D.R. Keeney, *Methods of soil analysis* (1982).
- [8]. M.L. Hair, W. Hertl, Reaction of hexamethyldisilazane with silica, *The Journal of Physical Chemistry*, 75, 2181-2185 (1971).
- [9]. Y. Sudo, Optimization of end-capping of octadecyl-silylated silica gels by high-temperature silylation, *Journal of Chromatography A*, 757, 21-28 (1997).