



ELSEVIER

Contents lists available at ScienceDirect

Talanta

journal homepage: [www.elsevier.com/locate/talanta](http://www.elsevier.com/locate/talanta)

# Preparation and chromatographic evaluation of zwitterionic stationary phases with controllable ratio of positively and negatively charged groups



Xiao-Dong Cheng, Yan-Hong Hao, Xi-Tian Peng, Bi-Feng Yuan, Zhi-Guo Shi, Yu-Qi Feng\*

Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), Department of Chemistry, Wuhan University, Wuhan 430072, China

## ARTICLE INFO

### Article history:

Received 11 February 2015  
Received in revised form  
23 March 2015  
Accepted 25 March 2015  
Available online 2 April 2015

### Keywords:

Hydrophilic interaction chromatography  
Zwitterionic stationary phase  
Controllable ratio of positively and negatively charged groups  
Retention mechanism

## ABSTRACT

The present study described the preparation and application of zwitterionic stationary phases (ACS) with controllable ratio of positively charged tertiary amine groups and negatively charged carboxyl groups. Various parameters, including water content, pH values and ionic strength of the mobile phase, were investigated to study the chromatographic characteristics of ACS columns. The prepared ACS columns demonstrated a mix-mode retention mechanism composed of surface adsorption, partitioning and electrostatic interactions. The elemental analysis of different batches of the ACS phases demonstrated good reproducibility of the preparation strategy. Additionally, various categories of compounds, including nucleosides, water-soluble vitamins, benzoic acid derivatives and basic compounds were successively employed to evaluate the separation selectivity of the prepared ACS stationary phases. These ACS phases exhibited entirely different selectivity and retention behavior from each other for various polar analytes, demonstrating the excellent application potential in the analysis of polar compounds in HILIC.

© 2015 Elsevier B.V. All rights reserved.

## 1. Introduction

In the early 1990s, hydrophilic interaction (liquid) chromatography (HILIC) emerged as an alternative approach to reversed-phase liquid chromatography (RPLC) for the separation of a wide range of analytes. It is becoming increasingly popular for the separation of polar compounds, such as polar pharmaceuticals, neurotransmitters, oligosaccharides, peptides, nucleosides, etc. [1–5]. Typically, this special LC mode combines stationary phases usually used in the normal phase (NP) mode and mobile phases for RP separations. It overcomes the drawbacks of the poor solubility encountered in NPLC and the poor retention in RPLC of polar compounds. Furthermore, it can be conveniently coupled to electrospray ionization (ESI) mass spectrometry (MS) and the higher organic content mobile phase in HILIC respect to RPLC significantly increases ESI-MS sensitivity because of improved ionization efficiency [6].

Along with increasing popularity of HILIC, a number of materials have been developed for HILIC. Most of them consist of classical bare silica or silica gels modified with various polar functional groups, such as diol [7,8], amino [9], amide [10] and other polar ligands [2,11,12]. Among those stationary phases, zwitterionic HILIC (ZIC-HILIC)

stationary phases, which were originally intended for ion-exchange separations, have been successfully utilized in HILIC. These kinds of phases contain both positively and negatively charged ligands, linked to silica or to a polymeric support. Typical zwitterionic stationary phases were prepared by introduction of zwitterionic compounds on the surface of silica particles [13,14]. Yin et al. [15] prepared a novel type of tridentate zwitterionic HILIC stationary phase by covalently bonding N-benzyl iminodiacetic acid on silica gel via copper (I) catalyzed Huisgen azide-alkyne 1,3-dipolar cycloaddition (CuAAC). Qiu et al. [16] proposed a new zwitterionic stationary phases by covalently bonding 3-p,p-diphenylphosphonium propylsulfonate to silica gel. In recent decades, ionic liquids (ILs) have been also utilized in the preparation of stationary phases due to their unique physico-chemical properties and designability of structure [17–20]. Qiao et al. [21] synthesized new zwitterionic stationary phases by binding an imidazolium ionic liquid 1-vinyl-3-(butyl-4-sulfonate)imidazolium to the surface of silica particles by “thiol-ene” click chemistry. The resulting stationary phases displayed excellent selectivity and efficient retention for various polar solutes. The zwitterionic functionalities give such stationary phases favorable water-retaining properties. These stationary phases have been reported to exhibit higher selectivity over some typical HILIC materials for the separation of polar analytes [3,21–24].

A sulfobetaine-based coating, which consists of positively charged quaternary ammonium and negatively charged sulfonic

\* Corresponding author. Tel./fax: +86 27 68755595.  
E-mail address: [yqfeng@whu.edu.cn](mailto:yqfeng@whu.edu.cn) (Y.-Q. Feng).

acid groups in a 1:1 ratio, is a typical zwitterionic phase and has a wide range of applications in HILIC [25]. As shown in Fig. 1a, the oppositely charged groups are distributed perpendicular to the silica with the sulfonic group at the distal end of the zwitterionic moiety. Another zwitterionic phosphorylcholine phase has also been reported previously [26]. It was prepared by graft polymerization of 2-methacryloyloxyethyl phosphorylcholine onto the surface of silica gel. As shown in Fig. 1b, the oppositely charged groups are also perpendicular to the silica but with different spatial arrangement of positive and negative charged groups. In addition to these zwitterionic phases, Shen et al. [14] developed a new type of cysteine-bonded stationary phase with uniform distribution of both positively and negatively charges which were parallel to the surface of the silica gel (Fig. 1c). This stationary phase exhibited high hydrophilicity and achieved successful separation of oligosaccharides, peptides and basic compounds. The major difference between those zwitterionic phases is the various functional groups and spatial arrangement of positive and negative charged groups, resulting in different surface charge and chromatographic behaviors.

Comparing with existing zwitterionic stationary phases, we propose tertiary amine–carboxyl-based silica (ACS) zwitterionic stationary phases. The tertiary amine groups cannot form Schiff bases with carbonyl compounds, which may result in improved lifetime with respect to typical aminopropyl silica. As shown in Fig. 1d, the oppositely charged tertiary amine and carboxyl groups are parallel to the surface of the silica. More importantly, the ratio of those two oppositely charged groups could be easily adjusted by changing the proportion of initial reactants. Such adjustable distribution of positively and negatively charged groups could provide an opportunity to tune the separation selectivity.

We prepared three types of ACS with different molar ratios of tertiary amine group to carboxyl group (4:1, 1:1 and 1:4). After characterization using elemental analysis, the influences of water content, pH, and buffer concentration in the mobile phase on retention behavior of the three ACS columns were investigated. Additionally, different kinds of test mixtures (nucleosides, water-soluble vitamins, benzoic acids, and basic compounds) were used

to compare the retention behavior and separation selectivity for the three types of ACS columns in the HILIC mode. The study provides an easy method for preparing zwitterionic stationary phases with controllable ratio of positively and negatively charged groups, indicating the great potential of ACS stationary phases in the separation of polar compounds in HILIC.

## 2. Experimental

### 2.1. Apparatus

The HPLC system used in this study is an Agilent HPLC series 1200 system (Agilent Technologies, Palo Alto, CA), which consists of a G1311A Quaternary pump, a G1315B DAD detector, a G1329A auto sampler, a G1330B thermostated column compartment, and a G1322A degasser. Elemental analysis was performed on a VarioEL III.

### 2.2. Chemicals and materials

Spherical silica (5  $\mu\text{m}$  particle size, 10 nm pore diameter and 300  $\text{m}^2\text{g}^{-1}$  specific surface area) was purchased from Fuji Silysia Chemical (Kasugai, Japan). Vitamins (B1, B2, B3, B3-amide, B6, B12), mercaptoacetic acid, dimethoxybenzoic acid, clenbuterol, and nucleosides were purchased from Aladdin (Shanghai, China). (N,N-Diethyl-3-aminopropyl)trimethoxysilane and vinyltriethoxysilane were obtained from Wuhan University Silicene New Material (Wuhan, China). Benzoic acid and its analogs, isopropanol, methanol (MeOH), triethylamine, acetonitrile (ACN), acetic acid, and ammonium acetate were purchased from Sinopharm Chemical Reagent Factory (Shanghai, China). ZIC-HILIC (250 mm  $\times$  4.6 mm i.d., 5  $\mu\text{m}$  and 20 nm pore diameter) was purchased from Merck SeQuant (Darmstadt, Germany). All other reagents were of analytical reagent grade unless otherwise indicated. Water used throughout the study was purified with a Milli-Q system (Millipore, Billerica, MA, USA).

### 2.3. Preparation of stationary phase and column packing

The ACS stationary phase synthesis procedure is shown in Fig. 2. Mercaptoacetic acid (2.76 g, 30 mmol), vinyltriethoxysilane (5.71 g, 30 mmol), and dimethoxybenzoic acid (0.15 g, 0.6 mmol) were mixed in a 20 mL borosilicate glass bottle, backfilled with nitrogen, and irradiated for 24 h with a 20 W blacklight ( $\lambda_{\text{max}}=365\text{ nm}$ ) to obtain the final carboxyl functional silanes [27]. The product was stored at  $-20^\circ\text{C}$  before its subsequent use. For ACS-1, 0.69 g of the obtained liquid (2.88 mmol), (N,N-Diethyl-3-aminopropyl)trimethoxysilane (2.39 g, 11.52 mmol) were mixed with silica (5 g) which was suspended in

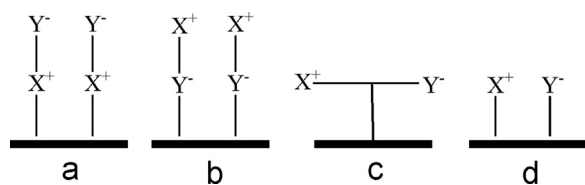


Fig. 1. Schematic of zwitterionic stationary phases.

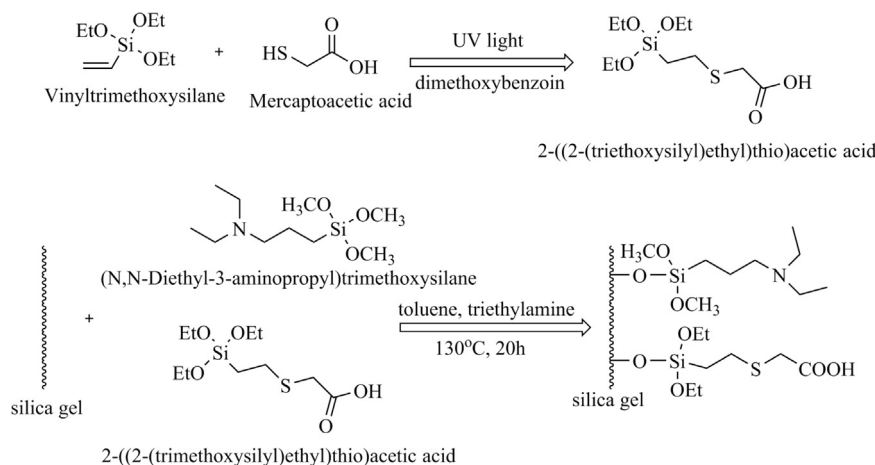


Fig. 2. The synthesis procedure of the ACS stationary phases.

Download English Version:

<https://daneshyari.com/en/article/7678468>

Download Persian Version:

<https://daneshyari.com/article/7678468>

[Daneshyari.com](https://daneshyari.com)