Journal of Chromatography A, 1557 (2018) 43–50

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

A facile and efficient single-step approach for the fabrication of vancomycin functionalized polymer-based monolith as chiral stationary phase for nano-liquid chromatography☆

Dongsheng Xu[a,b,c,1], Huikai Shao[a,1], Rongying Luo[a], Qiqin Wang[a], Elena Sánchez-López[c], Salvatore Fanali[d], Maria Luisa Marina[c,e,*], Zhengjin Jiang[a,b,**]

[a] Institute of Pharmaceutical Analysis, College of Pharmacy, Jinan University, Guangzhou 510632, China
[b] Department of Pharmacy and Guangdong Province Key Laboratory of Pharmacodynamic Constituents of Traditional Chinese Medicine & New Drug Research, Jinan University, Guangzhou 510632, China
[c] Departamento de Química Analítica, Química Física e Ingeniería Química, Universidad de Alcalá, Ctra. Madrid-Barcelona Km. 33.600, 28871 Alcalá de Henares, Madrid, Spain
[d] PhD School in Natural Science and Engineering, University of Verona, Strada Le Grazie, 15–37129 Verona, Italy
[e] Instituto de Investigación Química "Andrés M. del Río" (IQAR), Universidad de Alcalá, Ctra. Madrid-Barcelona Km. 33.600, 28871 Alcalá de Henares, Madrid, Spain

ARTICLE INFO

Article history:
Received 28 March 2018
Received in revised form 25 April 2018
Accepted 2 May 2018
Available online 3 May 2018

Keywords:
Vancomycin
Enantioseparation
Organic polymeric monolith
Nano-LC

ABSTRACT

A facile single-step preparation strategy for fabricating vancomycin functionalized organic polymer-based monolith within 100 μm fused-silica capillary was developed. The synthetic chiral functional monomer, i.e 2-isocyanatoethyl methacrylate (ICNEML) derivative of vancomycin, was co-polymerized with the cross-linker ethylene dimethacrylate (EDMA) in the presence of methanol and dimethyl sulfoxide as the selected porogens. The co-polymerization conditions were systematically optimized in order to obtain satisfactory column performance. Adequate permeability, stability and column morphology were observed for the optimized poly(ICNEML-vancomycin-*co*-EDMA) monolith. A series of chiral drugs were evaluated on the monolith in either polar organic-phase or reversed-phase modes. After the optimization of separation conditions, baseline or partial enantioseparation were obtained for series of drugs including thalidomide, colchicine, carteolol, salbutamol, clenbuterol and several other β-blockers. The proposed single-step approach not only resulted in a vancomycin functionalized organic polymer-based monolith with acceptable performance, but also significantly simplified the preparation procedure by reducing time and labor.

1. Introduction

Although a large number of chiral stationary phases (CSPs) are available on the market, the development of novel CSPs still attracts considerable interest [1,2]. Recently, increasing efforts have been directed toward the development of organic polymer-based chiral monolithic columns because of their excellent permeability, pH stability, low resistance to mass transfer and high performance [3–5]. So far, various chiral functionalized polymer-based chiral monoliths have been reported, such as cyclodextrin and its derivatives [6,7], quinidine and its derivatives [8–10], cellulose derivatives [11], proteins [12,13], macrocyclic antibiotics [14,15], crown ethers [16] and chiral ion-exchangers [17].

Over the years, the vancomycin-type glycopeptide antibiotics have been proved to be a versatile class of chiral selectors for enantioseparation in polar organic-phase, normal-phase and reversed-phase modes since their enantioselectivity was demonstrated by Armstrong et al. [18]. However, very few vancomycin functionalized polymer-based monoliths have been reported. So far, only Maruška and co-workers developed a multi-step post-column modification strategy for immobilizing vancomycin onto the surface of organic polymeric monolith at the turn of the century [14,15]. The polymeric support was prepared through *in situ* co-polymerization of *N*-(hydroxymethyl) acrylamide, allyl glycidyl ether and piperazine diacrylamide with vinyl sulfonic acid within

☆ Thematic Virtual Special Issue on "Enantioseparations-2018".

* Corresponding author at: Departamento de Química Analítica, Química Física e Ingeniería Química, Universidad de Alcalá, Ctra. Madrid-Barcelona Km. 33.600, 28871 Alcalá de Henares, Madrid, Spain.
** Corresponding author at: Institute of Pharmaceutical Analysis, College of Pharmacy, Jinan University, Guangzhou 510632, China.
E-mail addresses: mluisa.marina@uah.es (M.L. Marina), jzjjackson@hotmail.com (Z. Jiang).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.chroma.2018.05.004
0021-9673/

100 μm I.D. capillaries. Subsequently, vancomycin was introduced onto the polymeric skeleton *via* reductive amination of the aldehyde groups converted from epoxy groups. Later on, they simplified the preparation procedure by replacing allyl glycidyl ether with *N*, *N*'-diallyltartardiamide, which can be easily cleaved into two aldehyde groups using periodate treatment. The vancomycin functionalized polymer-based monoliths prepared through both ways exhibited good enantioselectivity for racemic compounds in capillary electrochromatography (CEC). However, the authors did not provide any column-to-column and batch-to-batch repeatability data in their studies. To the best of our knowledge, there is no other report about vancomycin functionalized polymer-based monoliths. This may be partially attributed to some disadvantages associated with multi-step preparation strategy, such as time-consuming, laborious and probably unsatisfactory repeatability. Vancomycin functionalized silica-based monoliths were also prepared through multi-step preparation strategy [19–22]. Hsieh et al. recently developed a single-step *in situ* sol-gel approach for preparing vancomycin functionalized silica-based monolith [23]. A sol-gel precursor containing vancomycin was synthesized and co-polymerized with skeleton precursor to form a porous silica-based monolith. The proposed single-step approach not only resulted in a chiral column with good efficiency and enantioselectivity for many basic enantiomers, but also significantly simplified the preparation procedure. Aiming at reducing the time and labor associated with the fabrication of vancomycin functionalized organic polymer-based monoliths, it would be of high interest to develop a single-step co-polymerization approach as well.

In this work, a chiral functional monomer, i.e 2-isocyanatoethyl methacrylate (ICNEML) derivative of vancomycin (ICNEML-vancomycin), was first synthesized. It was then *in situ* co-polymerized with the cross-linker ethylene dimethacrylate (EDMA) in a binary porogen system of methanol and dimethyl sulfoxide (DMSO). The polymerization conditions were systematically optimized in order to obtain satisfactory permeability, column efficiency and enantioresolution. The enantioresolution capability of the optimized monolith was evaluated by analyzing a series of chiral drugs in either polar organic-phase or reversed-phase modes. The enantioseparation conditions, including the organic solvent type and concentration, the buffer concentration and the pH of the mobile phase, were also carefully optimized.

2. Materials and methods

2.1. Reagents and samples

2,2′-azobisisobutyronitrile (AIBN), 3-(trimethoxysilyl)-propylmethacrylate (γ-MAPS), ethylene dimethacrylate (EDMA), 2-isocyanatoethyl methacrylate (ICNEML), DMSO, methanol (MeOH), ethanol, 1,4-butanediol, 1-propanol, tetrahydrofuran (THF), cyclohexane, 1-dodecanol and toluene, acetonitrile (ACN), triethylamine (TEA), acetic acid (HAc), pyridine, acetone and vancomycin hydrochloride were acquired from Aladdin Chemicals (Shanghai, China). Acebutolol, carteolol, sotalol, propranolol, pindolol, tertaolol, clenbuterol, salbutamol and thalidomide were obtained from Energy Chemical (Shanghai, China). Colchicine was purchased from Sigma (Missouri, MO, USA). The fused-silica capillaries (375 μm O.D. × 100 μm I.D.) were obtained from Ruifeng Chromatography Ltd. (Hebei, China). Distilled water was purified using a Milli-Q system (Massachusetts, MA, USA). Polar organic mobile phases were set up by mixing the desired ratio of ACN and MeOH, and then adding various amount of TEA and HAc. Reversed-phase mobile phases were prepared by mixing the corresponding ratio of MeOH or ACN and buffer containing TEAA.

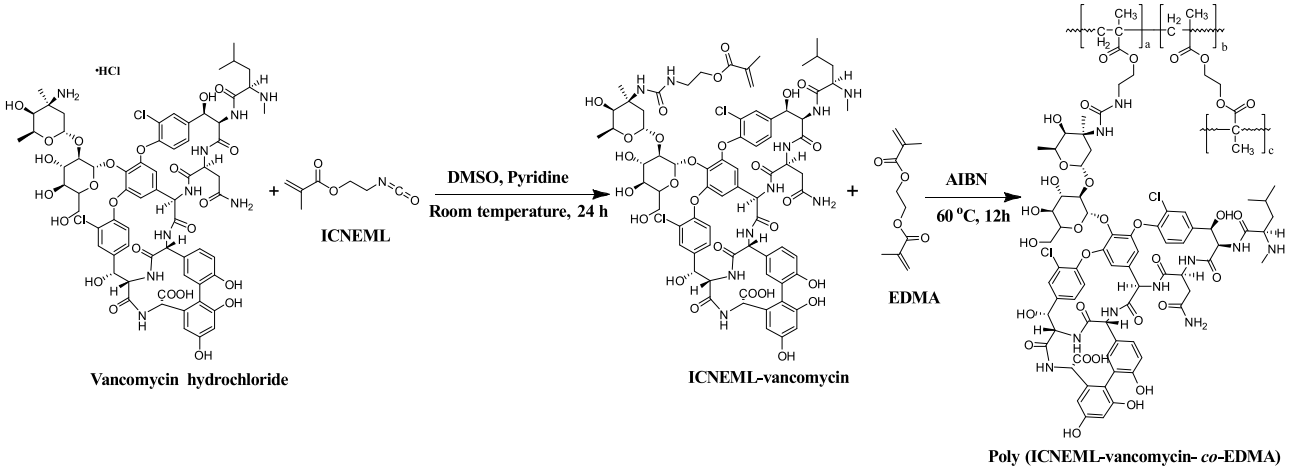

Fig. 1. Schematic representation of the synthesis of the ICNEML-vancomycin.

Table 1
Composition of the polymerization mixture used for the preparation of the poly (ICNEML-vancomycin-co-EDMA) monolith columns and their properties.

Column	Monomers (%, w/w)		Porogens (%, w/w)		Monomers: Porogens (%, w/w)		Backpressure (MPa)	Enantioresolution
	ICNEML-vancomycin	EDMA	MeOH	DMSO	Monomers	Porogens		
C1	75.0	25.0	75.0	25.0	29.0	71.0	Too high	/
C2	75.0	25.0	75.0	25.0	25.0	75.0	7.5	1.45
C3	75.0	25.0	75.0	25.0	21.0	79.0	3.4	0.37
C4	70.8	29.2	75.0	25.0	25.0	75.0	9.5	1.38
C5	79.2	20.8	75.0	25.0	25.0	75.0	3.6	0.51
C6	75.0	25.0	70.0	30.0	25.0	75.0	9.8	0.58
C7	75.0	25.0	80.0	20.0	25.0	75.0	4.7	0.94

Conditions column dimensions: 15 cm × 100 μm I.D.; mobile phase: MeOH/ACN/TEA/HAc (80/20/0.08/0.02, v/v/v/v); UV detection wavelength: 230 nm; total flow rate: 400 nL/min; injection volume: 20 nL; sample: acebutolol.