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Fabrication and evaluation of an organic monolithic column based upon the polymerisation of hexyl methacrylate with 1,6-hexanediol ethoxylate diacrylate for the separation of small molecules by capillary liquid chromatography

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ABSTRACT

This paper describes the fabrication of a new porous monolith, prepared in 100 μm i.d. capillaries by the co-polymerisation of hexyl methacrylate with 1,6-hexanediol ethoxylate diacrylate, poly (HMA-co-1,6 HEDA), in the presence of azobisisobutyronitrile, 1, 4-butanediol and 1-propanol were used as porogens for the monoliths; the monoliths were then used as a stationary phase for capillary liquid chromatography. Two cross linkers namely 1,6 HEDA and EDMA were utilised in order to investigate the effects of cross linker length on the separation efficiency of small molecules, and it was found that the efficiency of the separation improved tenfold when using the longer cross linker, 1,6 HEDA. This improvement is associated with the increase in number of methylene groups which resulted in an increased number of mesopores, less than 50 nm. The 1,6 HEDA based monolith showed a high porosity (90%) and no evidence of swelling or shrinking with the use of organic solvents. Moreover, the 1,6 HEDA monolith demonstrated high reproducibility for the separation of the retained compounds anisole and naphthalene; these showed retention time RSDs of 1.79% and 2.74% respectively. The fabricated monolith also demonstrated high selectivity for neutral non-polar molecules, weak acids, and basic molecules. The asymmetry factors for basic molecules (nortriptyline and amitriptyline) were 1.5 and 1.3 respectively, indicating slight tailing, which is often noticeable on silica based phases due to secondary interactions between basic moieties and the hydroxyl groups of the silica.

1. Introduction

Over the past decade, great interest has been directed towards micro-HPLC and its rapid development for various applications, including those in the life sciences, pharmaceutical industry, and environmental analyses. However, the inherent limitations of conventional packed columns, such as slow mass transfer, large void times and the difficultly of designing well packed columns, have simultaneously driven the growth of alternative separation materials such as monoliths [1].

Monolithic columns consist of continuous interconnected networks with large through-pores channels, and are an appealing substitute for conventional particulate stationary phases in capillary liquid chromatography; their structure leads to a decrease in the diffusion path and affords high permeability, which results in good separation efficiency. The core structure improves the mechanical strength, while the large through-pore channels possess very low flow resistance. Consequently, this combination allows for smaller diameter monolithic columns to be employed at high flow-rates, simultaneously increasing both sensitivity and throughput [2]. Moreover, the fabrication of monolithic columns in capillary formats has facilitated the coupling of micro-HPLC with the power of mass spectrometry to separate complex mixtures, for instance proteins and peptides [2].

Abbreviations: HMA, hexyl methacrylate; 1,6HEDA, 1,6-hexanediol ethoxylate diacrylate; AIBN, azobisisobutyronitrile; EDMA, ethylene dimethacrylate; γ-MAPS, 3-(trimethoxysilyl) propyl methacrylate; ROMP, ring-opening metathesis polymerisation; PEG, polyethylene glycol; SMA, stearyl methacrylate; PEGMEMA, poly (ethylene glycol) methyl ether methacrylate; AOD, 3-methylacryloyl-3-oxapropyl-3-(N,N-dioctadecylcarbamoyl)-propionate; PEDAS, pentaerythritol diacrylate monostearate; SEMA, 2-sulphoethyl methacrylate; 2,3,5TCP, 2,3,5-trichlorophenol; BMA, butyl methacrylate; LMA, lauryl methacrylate; BUDMA, tetramethylene dimethacrylate; HEDMA, hexamethylene dimethacrylate; SEM, scanning electron microscopy; RP-HPLC, reversed-phase chromatography; HILIC, aqueous normal phase liquid chromatography; R^2, correlation coefficient; TEA, triethylamine

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Polymer-based monoliths are commonly prepared by in situ polymerisation, employing either thermal initiation [3,4] or photochemical initiation [5] via free-radical cross-linking polymerisation. The alternative ring-opening metathesis polymerisation (ROMP) can also be used as a common approach to synthesise polymer-based monoliths [6–8]. ROMP employs a transition metal as catalyst for the polymerisation of the monolith [8].

Organic based monoliths are often characterised by a mono-model macropores pore size distribution and are therefore used for the separation of large molecules such as proteins and peptides. This limitation for organic monoliths is due to the low number of mesopores [5,9]. Previously, several approaches have been employed to allow the separation of small molecules on organic based monoliths including optimisation of polymerisation conditions by increasing or decreasing the temperature for initiation of the polymerisation process [10,11], changing monomers [12], and changing porogens [13]. However, these approaches did not improve the applicability of the organic monoliths to the desired degree [14,15]. More recently, several alternative approaches have been more successfully adopted in the literature to enhance the applicability of organic monoliths for the separation of small molecules. These include an early termination of the polymerisation process [16], using a single cross linker [17,18], applying of hyper-crosslinking [12], utilisation of carbon nanostructure [19], using longer cross linkers for reversed-phase chromatography (RP-HPLC) [20], or using more polar cross linkers when applying Hydrophilic Liquid Chromatography (HILIC) [21].

In the study described here, the effects of cross linker length on the separation efficiencies of small molecules have been investigated by utilising two cross linkers (shown in Fig.1a). The cross linkers used in this investigation were ethylene dimethacrylate (EDMA) and 1,6-hexanediol ethoxylate diacrylate (1,6 HEDA). The efficiency of the 1,6 HEDA based monolith was found to be superior to the EDMA based column, therefore, the porosity, permeability, and reproducibility of the 1,6 HEDA based monolith was further characterised. Furthermore, the effect of ACN content in the mobile phase on the retention factor and plate height for the retained molecules (anisole, naphthalene) was investigated for 1,6 HEDA based monolith. The plate height-flow velocity curve for the most retained molecule (naphthalene) was also examined. In addition, poly (HMA-co-1, 6HEDA) monolith was used for the separation of neutral non-polar molecules, weak acid molecules, and basic molecules to demonstrate the monolith's various applications.

2. Experimental

2.1. Reagents and materials

Hexyl methacrylate (HMA) was purchased from Lancaster, United Kingdom, 1, 6-hexanediol ethoxylate diacrylate (1,6HEDA) was

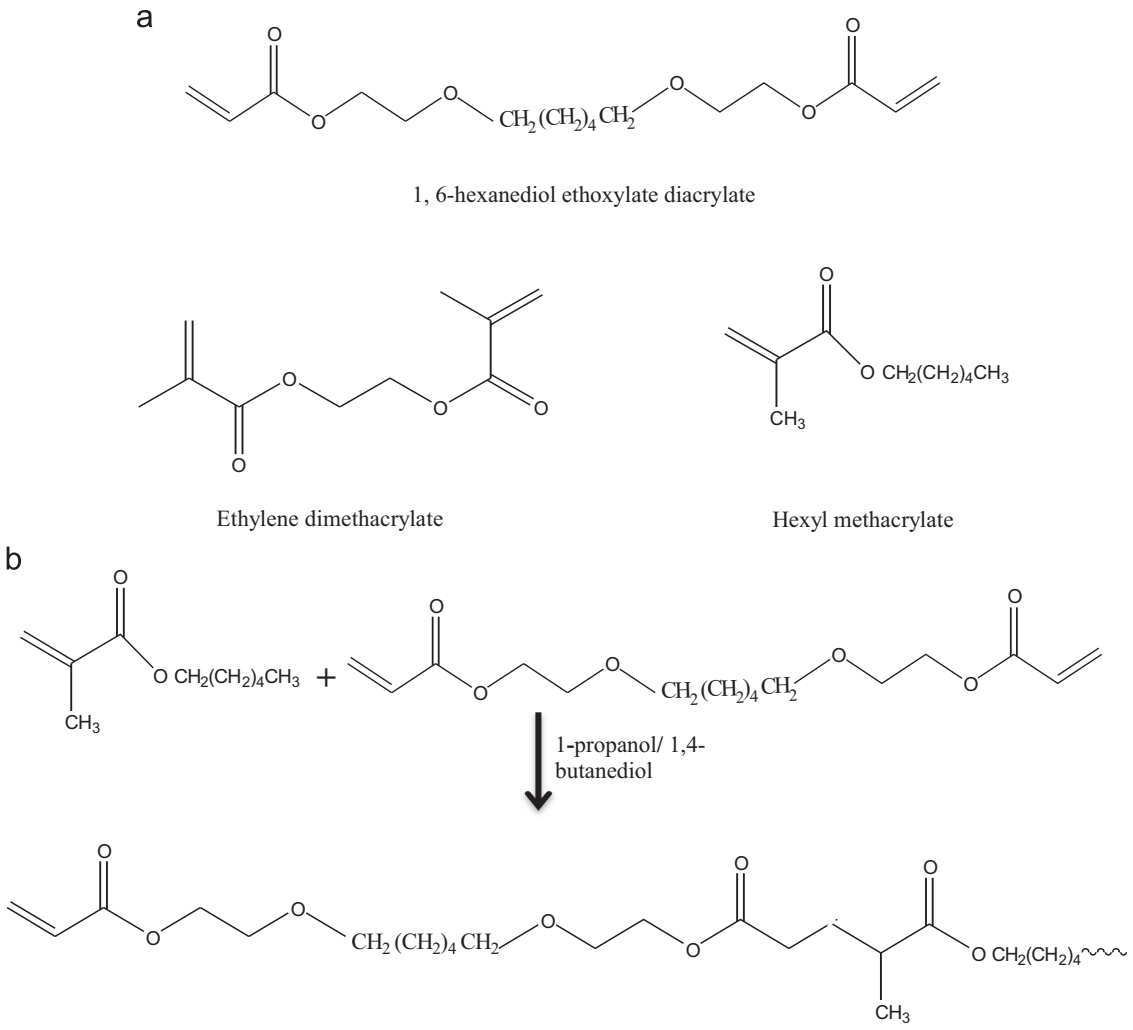

Fig. 1. (A) Chemical structures of monomer and cross-linkers used in the study, drawn by ChemDraw software pro version5, and (B) the co-polymerisation scheme of the selected monomers and porogens is demonstrated, drawn by ChemDraw software pro version5.