



Preparation of monodisperse porous poly (glycidylmethacrylate-co-ethylenedimethacrylate) microspheres and their application as stationary phase for superheated water HPLC

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ABSTRACT

Monodisperse porous poly(glycidylmethacrylate-co-ethylenedimethacrylate) (poly(GMA-co-EDMA) beads) with a diameter of 3 μm each were custom synthesized by a single-step swelling and polymerization method. Small sizes (1.2 μm) with polystyrene seed templates of low molecular weight were prepared by the addition of 1,1-diphenylethylene to control the polymerization reaction. Polystyrene seed particles were swollen directly by a mixture of methacrylate monomers and porogen. By swelling polystyrene seed particles with tetrahydrofuran as the porogen, a surface area of 108 $\text{m}^2 \text{g}^{-1}$ was achieved. The porous polymer microspheres were evaluated as stationary phase for liquid chromatography using superheated water as a mobile phase. Chromatographic performance at different temperatures was studied, along with analyte and column stability. The results confirm that the particles were stable under superheated water conditions. There was almost no change to the retention properties during the 160 sequential injections of a standard mixture (uracil, pyridine, phenol, dimethyl phthalate and *N,N*-dimethylaniline) at 150 °C. The chromatographic peaks show good symmetry with an efficiency of up to 47,000 plates m^{-1} under realistic separation conditions. Thermal decomposition of analytes (aldicarb sulfone, oxamyl, aldicarb and benomyl) separated under different column temperatures, was evaluate. Chromatograms of pesticide standard mixtures separated in the temperature range of 100–140 °C displayed good separation. The detection limits of 1.2 and 1.6 mg L^{-1} for benomyl and aldicarb were obtained when the separation was performed at 120 °C with flow rate of 0.4 mL min^{-1} water. However these pesticides were decomposed at the separation temperature above 140 °C.

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1. Introduction

Due to increasing pressure to develop more environmentally-friendly technologies, minimizing organic solvent usage, or replacing organic solvents with water is of great interest and has been studied by many researchers [1–3]. Water is a widely available, non-hazardous, and environmentally-friendly solvent. Water at ambient temperature is too polar to dissolve many organic compounds; but, at an elevated temperature, organic compounds become much more soluble due to the change in the polarity of water. Between 100 °C and its supercritical point at 374 °C, water is termed superheated water, subcritical water or pressurized water [4]. By selecting appropriate temperatures, water properties corresponding to different polarities can be generated.

Having obvious advantages in that it is green, cheap and safe [5,6], superheated water has been explored as a liquid

chromatography green mobile phase during this period [7–11]. In addition, several benefits for using high temperature liquid chromatography have also been reported [12–14]. An increase in temperature in typical reversed phase separations can cause a decrease in retention. In addition, decreased solvent viscosity at high temperatures leads to lower backpressure. This allows the use of higher flow rates while using standard equipment. Resolution and efficiency were also improved by using high temperature separations. Since the pressure drop decreased significantly due to an increase in temperature (largely due to reduced solvent viscosity), using longer columns to improve efficiency became a practical option.

Despite these advantages, widespread adoption of superheated water in liquid chromatography still faces a barrier regarding the stability of the stationary phase, which has hampered its wider uptake. The use of silica-based stationary phases in highly aqueous mobile phases and at high temperatures (above 80–90 °C) is limited due to the degradation thereof. Thus, several alternative materials have been used for superheated water chromatography, as reviewed by Smith [15] and Nawrocki [16]. Polymer based

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stationary phases have many ideal attributes, including high thermal stability. In most cases, polymer phases utilized in superheated water chromatography are based on styrene and divinylbenzene [17–20], but strongly hydrophobic characteristics make them unsuitable for reversed-phase separation of many analytes due to excessive capacity factors.

Polymer beads and monoliths that have relatively polar structures with respect to poly(styrene-co-divinylbenzene), in particular poly(glycidyl methacrylate-co-ethylene dimethacrylate), have been synthesized and used as packing materials for various types of chromatography [21]. While these materials have been applied in SEC and other modes of chromatography particularly for bio-molecules and also as microscale monoliths [22], they have never been investigated for elevated temperature liquid chromatography. The chromatographic performance of these relatively polar materials regarding the retention factor and the stability properties when used with pure water as mobile phase in high temperature chromatography have rarely been investigated.

Different techniques have been developed for the synthesis of porous monodisperse polymer particles for use as HPLC packing materials [23–27]. Classical suspension polymerization is simple and well-established, but provides broad particle size distribution beads. Narrow size distribution particles can be conveniently prepared by precipitation polymerization, but they can be difficult to functionalize by copolymerization due to the effect it has on the balance between monomer and polymer salvation. The multistep swelling method established by Ugelstad et al. in the 1980s [28] provides a route to monosized polymer beads where the size is no longer determined by the stirring conditions or droplet size but by the swelling of the preformed uniform particles. These particles called “seeds” or “shape templates”, increase their size in a uniform fashion during swelling processes to provide monodisperse polymer beads. An aqueous dispersion of seeds is swollen to the target size of the final product with a mixture that may contain combinations of solvents, monomers, and crosslinking agents. The swollen particles are then polymerized. Although the shape and size uniformity of seed particles have been preserved, their size is increased significantly. With the presence of a solvent called a “porogen” or “porogenic agent” during the polymerization, a porous structure can be formed. Ogino et al. [29] reported a single step swelling and polymerization method in 1995 for preparing uniform styrene-co-divinylbenzene (poly(S-co-DVB)) beads in the size range of 4.1–7.5 μm . Only one step swelling was used; polymer seed particles were swollen directly by a monomer and porogen mixture [30]. This method makes preparation of monodisperse polymer particles easier.

In this study, monodisperse porous polymer particles were synthesized by the admixing of glycidylmethacrylate (GMA) together with ethylenedimethacrylate (EDMA) crosslinking agent using a single step swelling and polymerization method. Poly (GMA-co-EDMA) particles were chosen to used as stationary phase as these particles show more hydrophilic which provides lower retention factor for hydrophobic or apolar analytes compared to that of poly(EDMA) and poly(S-co-DVB). Polystyrene seed particles were swollen by using a mixture of methacrylate monomers and porogen. Diphenylethylene was added during the synthesis of polystyrene seeds [31] in order to control the shape and size of the resulting polymer particles and provide the optimum size range for efficient chromatography. Due to the green chemical benefits and the physico-chemical benefits of elevated temperature HPLC, the application of these porous polymer particles as a stationary phase was evaluated using pure water as mobile phase. Retention properties, peak shape, column efficiency and stability were studied. A standard test mixture comprising uracil, pyridine, phenol, dimethyl phthalate and N,N-dimethylaniline was investigated. In addition, the applicability of such materials in a pesticide

separation was evaluated, which highlighted an issue of analyte stability for one of the analytes studied during high temperature separation above 140 °C.

2. Experiment section

2.1. Chemicals

Styrene monomer was extracted with a NaOH (1 M) solution in order to remove inhibitors. Glycidyl methacrylate (GMA) and ethylene dimethacrylate (EDMA) were used without purification. All monomers and crosslinkers were obtained from Sigma-Aldrich (Poole, UK). Benzoyl peroxide (BPO), from Lancaster Synthesis (Morecambe England), recrystallized from ethanol, were used as oil-soluble initiators. 1, 1-Diphenylethylene (DPE) was supplied by Fisher Scientific (Leicestershire, UK) and used as reaction controller for seed preparation. Sodium dodecyl sulfate (SDS) from Sigma-Aldrich (Poole, UK) was used as emulsifier in the preparation of an aqueous emulsion medium for the swelling of seed particles. Polyvinylpyrrolidone (PVP K-90) from Fluka (Buchs, Switzerland) was used as stabilizer. Tetrahydrofuran (THF) from Fisher Scientific was used as porogen. The mixture used for superheated water chromatography testing consisted of pyridine from BDH chemicals (Poole, UK), uracil, phenol, dimethyl phthalate, and N,N-dimethylaniline obtained from Sigma-Aldrich. The pesticide mixture was prepared from aldicarb from Sigma-Aldrich, aldicarb sulfone and benomyl from Labor Dr. Ehrenstorfer (Augsburg, Germany) and oxamyl from Chem Service (West Chester, USA). Ultrapure water from Simplicity[®] (Merck Millipore, Germany) with resistivity of 18.2 M Ω cm) was used as the mobile phase.

2.2. Preparation of polystyrene (PS) seed particles

Dispersion polymerization [32,33] for the preparation of PS seed particles was modified. The mixture of 12.5 mL of styrene containing 0.625 g of PVP K-90 in 20 mL of ethanol was placed in a four-necked flask. When the temperature reached 75 °C, 0.30 g of BPO in 5 mL of ethanol was injected into the reaction. After the reaction had proceeded for 1.5 h, 2.0% diphenylethylene (DPE) (0.25 g), based on monomer concentration, was employed in 25 mL of ethanol to control the reaction [31]. The reaction was allowed to proceed for 24 h. The PS seed product was purified by a typical centrifugal method with at least 3 repeats of separation and re-dispersion in a fresh medium.

2.3. Preparation of porous poly(GMA-co-EDMA)

To produce porous poly(GMA-co-EDMA) particles, an initiator, 0.15 g of BPO, was dissolved in a homogeneous organic phase containing 4 mL of tetrahydrofuran (THF), 2.4 mL of GMA and a 1.6 mL EDMA monomer. The resulting organic phase was emulsified in an aqueous medium of 80 mL of water consisting of 0.08 g of SDS and 0.4 g of PVP K-90 by an ultra-turrax homogenizer for 1 min. The aqueous dispersion of 0.2 g of PS seed was transferred to the emulsion. The new dispersion was stirred magnetically at 125 rpm for 24 h. Polymerization of the monomer phase in the swollen seed particles was carried out in the reactor at 75 °C for 24 h under N₂ with a stirring rate of 100 rpm.

2.4. Polymer characterization

A PL-GPC 220 integrated GPC system (Varian, USA) connected to a refractive index detector was operated at 40 °C to measure the molecular weight of the PS samples. To transform all epoxy groups