Preparation, Characterization and Applications of Electron-Beam Curing-Derived Monolithic Materials

Rajendar Bandari,[1] *Wolfgang Knolle,*[1] *Michael R. Buchmeiser**[1,2]

Summary: Acrylate/methacrylate-based monoliths have been prepared *via* electron-beam (EB) initiated polymerization. These monolithic columns were found suitable for application in the analytical scale separation of various analytes such as proteins. Functionalization of the monolithic columns was accomplished *via* copolymerization of functional monomers. Selected examples of both synthesis and application will be summarized.

Keywords: electron beam curing; monolithic materials; radical polymerization; separation science

Introduction

Monolithic media nowadays hold a strong place in separation science and heterogeneous catalysis.[1–20] So far, polymer-based monoliths have been prepared *via* free radical polymerization,[13] controlled, TEMPO-initiated radical[21–23] or living metathesis polymerization,[11,19,24–36] polycondensation[37] and polyaddition[38] reactions. In this context, radical polymerization based syntheses have been triggered thermally, or *via* UV or γ-irradiation.[39,40] Here, we wish to summarize our latest results on the electron-beam (EB) triggered synthesis of polymeric monolithic materials, their functionalization and use in separation science.

Experimental Part

All manipulations were performed under an argon atmosphere in an MBraun glove box or by Schlenk techniques. Narrow polystyrene (PS) standards with molecular masses (M_w) of 972, 2600, 4000, 6810, 17200, 803000, 3390000 and 4110000 g/mol were purchased from Waters (USA). In addition, polystyrene standards with molecular masses (M_w) of 4000, 30000, 200000, and 400000 g/mol were purchased from Polymer Standards Service (PSS, Mainz, Germany). Chloroform (99.5%) and 2-propanol were purchased from KMF Laborchemie Handels GmbH (Germany) and dried over CaH_2 prior to use. Trimethylolpropane triacrylate (TMPTA), ethyl methacrylate (EMA), 3-(trimethoxysilyl) propyl methacrylate, 1-dodecanol, acetonitrile, water (HPLC-grade), trifluoroacetic acid (99.5%), ribonuclease A (from bovine pancreas, M_w = 13,700 g/mol), lysozyme (from chicken egg white, M_w = 14,307 g/mol), albumin (from human serum, Mw = 66,478 g/mol), insulin (from bovine pancreas, M_w = 5,733 g/mol), cyctochrome C (from bovine milk, M_w = 13,000 g/mol) and peptide separation buffer (PSB) were purchased from Sigma-Aldrich Co, Germany. Toluene and methylene chloride were dried by a MBraun SPS system. For inverse SEC (ISEC), a L-4500 UV detector (Merck), a L-6200A HPLC pump (Merck) equipped with a manual sample injection system were used. A D-7000 HPLC system was used for data acquisition and processing. All standards were prepared by dissolving the peptides of interest in the peptide separation buffer (PSB) followed by further dilution with

[1] Leibniz-Institut für Oberflächenmodifizierung (IOM) e. V., Permoserstr. 15, D-04318 Leipzig, Germany E-mail: michael.buchmeiser@iom-leipzig.de
[2] Institut für Technische Chemie, Universität Leipzig, Linnéstr. 3, D-04318 Leipzig, Germany

mobile phase and stored at −20 °C. HPLC-measurements were carried out by the use of a SCL-10 AVP system controller, a LC-10AT HPLC pump, a SIL-10A auto injector, a SPD-M10AVP diode array detector and a CTO-10AC column oven and a CLASS-VP503 software (all by Shimadzu). Eluents were degassed with He. ICP-OES measurements were carried out using Spectro ciros vision (Spectro Analytical Instruments GmbH&Co, Germany). Electron microscopy (SEM) was carried out with a Ultra 55 (Carl Zeiss SMT, Oberkochen, Germany).

Preparation of Monoliths

All monoliths were prepared as follows. Stainless steel columns (150 × 4.6 mm i.d.) were cleaned, rinsed and sonicated in a 1:1 mixture of ethanol and acetone. Columns (3 × 100 mm) used for protein separation were extensively washed with ethanol and deionized water to remove any surface impurities, immersed in an aqueous 0.2 mol l^{-1} sodium hydroxide solution overnight and then washed with water. Once the pre-treatment was completed, glass columns were rinsed with ethanol and dried at 110 °C for 4 h in an oven. For silanization, glass columns were then treated with a 50 wt.-% solution of 3-(trimethoxysilyl) propyl methacrylate in toluene at 40 °C for 16 h. The columns were rinsed with 3 mL of toluene and acetone, respectively, and dried for 2 h *in vacuo*. Stainless steel columns were closed at one end with frits and end fittings, respectively, glass columns were placed inside a test tube. The columns were filled with the polymerization mixture, sealed at either side and irradiated. Unless stated otherwise, a total dose of 22 kGy was applied. After irradiation, the columns were directly connected to a HPLC pump and flushed with chloroform for 4 h at a flow rate of 0.2 mL/min. After this procedure, the monoliths were ready for use and characterization.

Characterization of Porous Properties

Characterization was accomplished applying ISEC according to a published procedure.[42,43] For the measurements of retention times, 10 μL samples of individual polystyrene standards (0.25 mg/mL), dissolved in the mobile phase (chloroform), were injected onto each column applying a flow rate of 0.6 mL/min. For the determination of the total accessible porosity of the column, a 10 μL sample of benzene was injected. All chromatograms were recorded at a wavelength of 254 nm. Retention times and volumes corresponding to each injection were determined from the peak maximum. All retention volumes were corrected for the extra-column volume of the equipment. Calculations were carried out assuming that the hydrodynamic radii of the PS standards in $CHCl_3$ do not differ significantly from those reported in CH_2Cl_2.[42,43] Monoliths were characterized by inter-micro globule porosity (ε_z), micro porosity (ε_P), total porosity (ε_t), pore volume (V_p, mL/g), mean particle diameter (d_p, nm), mean pore size (Φ_m, Å) and specific surface area (σ).

General Procedure for EB Irradiation

Electron-beam irradiation was performed at the 10 MeV linear accelerator ELEKTRONIKA (Toriy Company, Moscow). The accelerator was operated at a 50 Hz repetition rate and 4 μs pulse length using a scanning horn (width up to 40 cm, scanning frequency 1 Hz) and a movable table to irradiate the samples. As a reference dose, the dose to graphite was determined with a calorimeter without further correction for the particular material irradiated. The total dose was applied incrementally in small steps (~3 kGy) over a period of 15 minutes in order to keep the temperature increase well below 50 °C.

Protein Separation

Monolith recipes: EMA:TMPTA:2-PrOH:1-dodecanol:toluene = 15:15:30:30:10 (all wt.-%). The irradiation dose was 22 kGy. Column dimensions: 3 × 100 mm; mobile phase A: 95% water + 5% acetonitrile + 0.1% TFA; mobile phase B: 20% water + 80% acetonitrile + 0.1% TFA; linear gradient, 10–90 % B in 2.0 min.; flow rate, 3 mL/min; T = 25°C; detection, UV

(200 nm). Elution order: (1) lysozyme, (2) ribonuclease A, (3) insulin, (4) cytochrome C, and (5) albumin. Injection volumes of proteins 1, 3-5 were 2.5 μg and for 23 μg.

In Situ Polymerization of Functional Monomer

A stainless steel column (150 × 4.6 mm i.d.) was closed at one end with frits and end fittings and the column was filled with the polymerization mixture consisting of EMA (15 wt.-%), TMPTA (15 wt.-%), 2-propanol (30 wt.-%), 1-dodecanol (30 wt.-%), toluene (10 wt.-%) and the N, S or halogen-containing functional monomer (0.5 wt.-%). The column was sealed and irradiated (22 kGy). After irradiation, the column was directly connected to a HPLC pump, flushed with chloroform for 4 h at a flow rate of 0.2 mL/min, then with THF for a further 30 min. Grafting yields were determined via elemental analysis quantifying N, S or halogen.

Results and Discussion

Monolith Synthesis *via* EB Curing

For EB curing, no initiator is required since the formation of radicals is simple triggered by the electron beam. So far, we used ethyl methacrylate (EMA) and trimethylopropane triacrylate (TMPTA) as monomers. 2-Propanol (2-PrOH) and 1-dodecanol were used as macroporogens, toluene as a microporogen. Preliminary experiments carried out with various concentrations of the components revealed that EMA and TMPTA concentrations of 15-25 wt.-% were suitable to induce phase separation. In this case, the 2-PrOH as well as the 1-dodecanol concentration was varied in the range of 20-30%. The concentration of the microporogen, toluene, was set to 10 wt.-% throughout. A selection of the composition of the monoliths is given in Table 1.

From simple curing measurements, a final dose of 22 kGy applied over a period of 15 minutes, giving raise to approximately 70–90% conversion, was identified to be sufficient for the curing of a polymerization mixture containing 45 or 50 wt.-% of EMA and TMPTA (1:1 wt.-ratio) kept within the confines of a 3 × 100 mm column while preserving the required low back pressure. Washing of the monoliths eluted significant amount of monomers and oligomers and resulted in porous monolithic structures that were well suited for our purposes.

Structural Characterization

A structural characterization of monoliths was performed by electron microscopy (SEM). Figure 1 shows some of the structures obtained.

One can clearly see the macroporous structure and the structure forming microglobules with average diameters in the sub micrometer range. In addition, inverse size exclusion chromatography (ISEC)[42,43] was applied. These measurements revealed that a mainly macroporous structure with a low percentage of pores <100 Å had been created. However, the monolithic structures can in fact, depending on their composition, be varied resulting in specific

Table 1.
Composition (in wt.-%) of monoliths **1–4**.

#	EMA	TMPTA	2-PrOH	1-Dodecanol	toluene	dose (kGy)
1[a)]	15	15	30	30	10	22
2[b)]	20	20	25	25	10	22
3[b)]	25	25	20	20	10	22
#	EMA	TMPTA	2-PrOH	1-dodecanol	toluene	functional monomer
4[b)]	15	15	30	30	10	0.5 (22 kGy)

[a)] Glass columns (3 × 100 mm);
[b)] Stainless steel column (4.6 × 150 mm). functional monomer: N,N-dipyridin-2-yl-methacrylamide.[41]

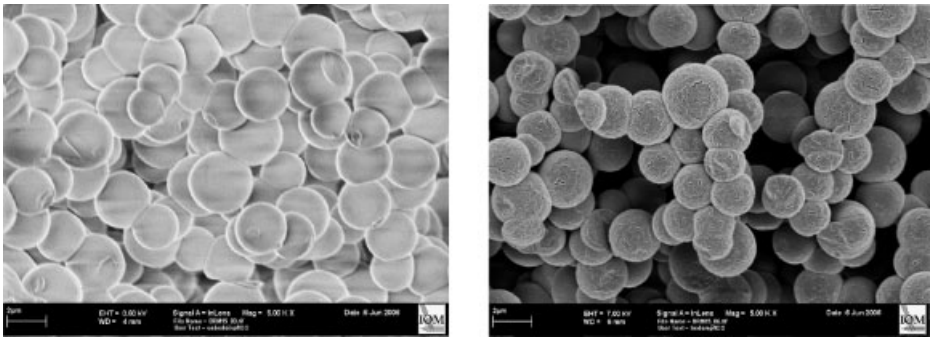

Figure 1.
Selected SEM pictures of EB-curing derived monoliths.

surface areas (σ) in the range of 15–23 m^2/g, pore volumes (V_p) between 60 and 216 μL/g and total porosities (ε_t) in the range of 44 to 83%.

Application in Separation Science

Monoliths 3 × 100 mm in size were used for the separation of a mixture of five proteins, i.e. lysozyme, ribonuclease A, insulin, cytochrome C, and albumin. Semi-preparative separation of these compounds was achieved on a 3 × 100 mm i.d. column within less than 100 s applying gradient elution (Figure 2). Peak half widths ($\omega_{0.5}$) were <3 s, resolution (R_s) was >0.8 throughout.

Functionalization and Application in Heterogeneous Catalysis

The general applicability and versatility of monolithic supports in heterogeneous catalysis has been demonstrated during the last years.[10,44] More recent applications were found in the areas of bioreactors,[45,46] scavenging materials,[47–50] or as supports for metal clusters. In this context, particularly ring-opening metathesis polymerization(ROMP)-derived monolithic supports[11] have been extensively used for the immobilization of Pd-based Heck catalysts,[15] Schrock[20] and Grubbs catalysts.[14,16–18,20,51] Both continuous flow

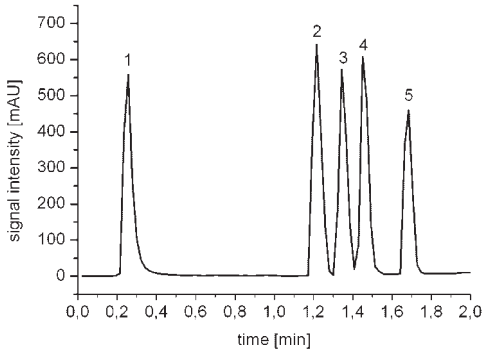

Figure 2.
Separation of a protein standard on an EB-derived monolith. Column dimensions: 3 × 100 mm, peak order (1) lysozyme, (2) ribonuclease A, (3) insulin, (4) cytochrome C, (5) albumin. mobile phase A: 95% water + 5% acetonitrile + 0.1% TFA; mobile phase B: 20% water + 80% acetonitrile + 0.1% TFA; linear gradient, 10–90% B in 2.0 min.; flow rate, 3 mL/min; T = 25 °C; detection, UV (200 nm).

devices[14,16–18] as well as monolithic discs[20,51] were manufactured.

Functionalization of EB-derived monolithic supports was accomplished via copolymerization of functional monomers, e.g. N,N-dipyrid-2-ylmethyacryl amide (Table 1). Such an approach is straightforward and always results in a more or less continuous distribution of the functional monomer within the monolithic support. Loading with Pd (II) resulted in a monolithic continuous flow reactor with a final Pd- loading of 7.8 mg/g. It was used to demonstrate its feasibility in heterogeneous catalysis. A simple Heck coupling of iodobenzene with styrene was used to monitor reactivity and determine the leaching of palladium. A constant, almost quantitative conversion (>95%) was observed over a range of 5 hours. The highly macroporous nature of the support facilitated mass transfer and gave rise to a low back pressure (<1.5 MPa @ a flow rate of 0.7 cm/min). Low amounts of palladium leached into the reaction mixture (4.2%). A ratio of *trans:cis* stilbene of 93:7 was found, characteristic for the dipyridylamide ligand.[52]

Conclusion

An electron beam (EB) based process for the manufacture of small and large-diameter monolithic materials has been developed. The novel monoliths are applicable to separation science as well as to heterogeneous catalysis. So far, functionalization of the novel devices was accomplished *via* copolymerization of a functional monomer.

Acknowledgements: Our work was supported by a grant of the *Deutsche Forschungsgemeinschaft* (DFG, BU2174/1-1) and by the *Freistaat Sachsen*.

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