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Ring-opening metathesis polymerization-derived monolithic anion exchangers for the fast separation of double-stranded DNA fragments

Said H. Lubbad[a,c,*], Michael R. Buchmeiser[a,b,**]

[a] Lehrstuhl für Makromolekulare Stoffe und Faserchemie, Institut für Polymerchemie, Universität Stuttgart, D-70569 Stuttgart, Germany
[b] Institut für Textilchemie und Chemiefaser Denkendorf (ITCF), Körschtalstr. 26, D-73770 Denkendorf, Germany
[c] Department of Chemistry, Al-Azhar University, Gaza, Palestine

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ABSTRACT

Ring-opening metathesis polymerization- (ROMP) derived monoliths were prepared from 5-norborn-2-enemethyl bromide (NBE-CH_2Br) and tris(5-norborn-2-enemethoxy)methylsilane ((NBE-$CH_2O)_3SiCH_3$) within the confines of surface-silanized borosilicate columns (100 × 3 mm I.D.), applying Grubbs' first generation benzylidene-type catalyst [$RuCl_2(PCy_3)_2$(CHPh)]. Monoliths were converted into weak anion exchangers via reaction with diethyl amine. The resulting monolithic anion exchangers demonstrated a very good potential for the anion-exchange separation of nucleic acids applying a phosphate buffer (0.05 mol/L, pH 7) and NaCl (1.0 mol/L) as a gradient former. Fast and efficient separations, indicated by sharp and highly symmetric analyte peaks, were established. Except for the 267 and 298 base pair fragments, the eleven fragments of a *ds*-pUC18 DNA Hae III digest were baseline separated within ~8 min. Nineteen fragments of a *ds*-pBR322 Hae III digest were separated within ~12 min. There, only the 192 and 213 base pair fragments and the 458, 504 and 540 base pair fragments coeluted. A *ds*-pUC18 DNA Hae III digest was used as a control analyte in evaluating the influence of organic additives on the mobile phase such as methanol and acetonitrile on nucleic acid separation. Methanol, and even better, acetonitrile improved the separation efficiency and shortened the analysis time.

1. Introduction

Since their discovery about two decades ago, monolithic columns have gained high popularity in separation science [1]. They allow for fast separations, which are a consequence of the high linear flow (up to 20 mm/s) through the support and the fast mass transfer between the stationary and the mobile phase [2,3]. Depending on their porosity and pore size distribution, monoliths have proven to be excellent chromatographic supports for the separation of a large variety of analytes, including small, i.e. low molecular weight molecules, and peptides, proteins and nucleic acid [4–12].

So far, the sizing of double-stranded (*ds*-) DNA was achieved by reversed-phase high-performance liquid chromatography (RP-HPLC) [13–17], anion-exchange high-performance liquid chromatography [18–20] or by capillary electrophoresis (CE) [21,22]. Comparably long analysis times and the small sample capacity still present drawbacks in capillary electrophoresis for such a separation. However, applying RP-HPLC, styrene-, acrylate-based [14–17] and ring-opening metathesis polymerization- (ROMP) derived monoliths [13,23,24] demonstrated good efficiency in the separation of nucleic acids. Nevertheless, RP-HPLC entails the use of toxic organic solvents (mainly acetonitrile). Consequently, anion exchange-chromatography appears attractive due to the benign, mainly water-based mobile phases used. Because of the different formats available (discs), however, the separation performance for nucleic acids of acrylate-based monoliths with anion exchange functionality [25–31] is until now not competitive with the one achieved in RP-HPLC and CE.

ROMP-derived polymers are a unique and competitive alternative to both polystyrene and poly(acrylate)-based monoliths [7,13,32,33]. Thus, this type of monoliths demonstrated excellent efficiency of nucleic acid on the microscale separation in RP-HPLC mode [13]. Usually, functionalization of this type of monoliths is performed via surface grafting of a functional monomer taking advantage of the living nature of the initiator used in monolith synthesis [32,33]. This way, both anion [34] and cation exchangers [35] were prepared. Nevertheless, we probed an alternative and more straightforward method for functionalization of these types of monoliths aiming on high column ion-exchange capacity. In this

* Corresponding author.
** Corresponding author at: Institute of Polymer Chemistry, University of Stuttgart, Pfaffenwaldring 55, D-70569 Stuttgart, Germany. Tel.: +49 0711 68564075; fax: +49 0711 68564050.
E-mail addresses: said.lubbad@ipoc.uni-stuttgart.de (S.H. Lubbad), michael.buchmeiser@ipoc.uni-stuttgart.de (M.R. Buchmeiser).

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Table 1
Fragment size as a number of base pairs of [I] a pUC 18 DNA Hae III digest and [II] a pBR 322 DNA Hae III digest separated by anion exchange on a ROMP-derived monolith.

Fragment	[I]	[II]
1	11	8
2	18	11
3	80	18
4	102	21
5	174	51
6	257	57
7	267	64
8	298	80
9	434	89
10	458	104
11	587	123
12		124
13		184
14		192
15		213
16		234
17		267
18		434
19		458
20		504
21		540
22		587

study, an easy access to ROMP-derived monolith with weak anion exchange capacity is described and their use in the separation of nucleic acids is presented.

2. Experimental

2.1. Chemicals and reagents

HPLC-analyses were performed using HPLC-grade water, methanol and acetonitrile (ACN) purchased from Sigma–Aldrich (Germany). NaH_2PO_4 and NaOH, (Sigma, Germany) were used for buffer preparation. A NaH_2PO_4 buffer (0.05 M) with different organic additives was prepared by adjusting the solution with NaOH to the required pH. The *ds*-pUC 18 DNA Hae III digest and *ds*-pBR322 DNA Hae III digest were purchased from Sigma-Life Sciences (Germany). The number and size of fragments present in these two samples are listed in Table 1. Tris-(5-norborn-2-enemethoxy)methylsilane ($(NBE\text{-}CH_2O)_3SiCH_3$) was prepared from 5-norborn-2-enemethanol and trichloromethylsilane (Sigma, Germany) [36]. These two compounds were used as the monomer and crosslinker, respectively. 2-Propanol, toluene Na_2CO_3 and diethyl amine were purchased from Sigma–Aldrich. Polystyrene standards $580 < M_n < 2{,}750{,}000$ g/mol, used for inverse size exclusion chromatography (ISEC) [37,38], were purchased from Polymer Standards Service, PSS (Germany).

2.2. Instrumentation

An Agilent Technology HPLC-system (Germany) was used for the analyses and separation of nucleic acids. The system consisted of a binary HPLC pump, a diode array UV–vis detector, an autosampler, a column oven and a sample thermostat. Nucleic acids were detected at $\lambda = 254$ nm. An Agilent GC–MS was used for the kinetic study of monomer polymerization. Borosilicate glass columns (Chromsep glass column, (100×3 mm I.D.), catalogue number CP99912) were purchased from CP-Analytica (Vienna, Austria). NMR data were obtained at 250.13 MHz for proton and 62.90 MHz for carbon in the indicated solvent at 25 °C on a Bruker Spectrospin 250 and are listed in parts per million downfield from tetramethylsilane for proton and carbon. Coupling constants are listed in Hz.

2.3. Preparation of 5-norborn-2-enemethyl bromide ($NBE\text{-}CH_2Br$)

This compound was prepared by the Diels-Alder reaction of allyl bromide with cyclopentadiene (freshly cracked from dicyclopentadiene at 180 °C). The crude product was purified by vacuum distillation and the pure product was collected as a colorless liquid at $T = 60$ °C. Yield: 78%, *exo*/*endo* mixture. ^{1}H NMR ($CDCl_3$): $\delta = 5.99$–6.22 (m, 2H, C=C), 3.47–3.02 (m, 2H, CH_2Br), 2.99 (m, 1H, CH), 2.88 (m, 1H, CH), 2.52–2.49 (m, 1H, CH_2), 1.96–1.84 (m, 1H, CH), 1.48 (m, 1H, CH), 1.29 (m, 1H, CH), 0.61–0.58 (m, 1H, CH); ^{13}C NMR ($CDCl_3$) $\delta = 138.3$, 137.1, 136.4, 131.6 (CH), 49.7 (CH_2), 45.5 (CH), 45.0, 42.2 (CH), 39.3, 38.3 (CH_2-Br), 33.4, 32.8 (CH_2); GC–MS (EI) m/z calculated for $C_8H_{11}Br$, $m/z = 186.00$; found: 186.0 (*exo*/*endo* mixture, $t_{R1} = 6.428$ min; $t_{R2} = 6.474$ min).

2.4. Monolith synthesis and functionalization

Borosilicate glass columns (100×3 mm I.D.) were etched at 60 °C with 2 M ethanolic KOH overnight. After repeated washing with water, columns were dried *in vacuo*. Silanization was performed at 60 °C overnight using a mixture of pyridine, toluene and bicyclo[2.2.1]hept-2-ene-5-methyldichlorosilane (molar ratio 3:2:1). Then, these columns were washed consecutively with acetone, water, and ethanol and dried under reduced pressure at ambient temperature.

Monoliths were prepared according to a previously described protocol (Fig. 1) [13]. Briefly, the glass column was capped with a 2-mL Eppendorf vial, placed in an ice-water bath and cooled to 0 °C. Two different solutions (A, B) were prepared using Schlenk techniques and cooled to −30 °C. Solution A consisted of $NBE\text{-}CH_2Br$, $(NBE\text{-}CH_2O)_3SiCH_3$ and 2-propanol (25:25:41.9 wt.%); solution B consisted of toluene and the initiator (7.7:0.4 wt.%). Solutions A and B were merged and mixed for few seconds. The polymerization mixture was then filled into the sealed borosilicate column. The column was then kept at 0 °C for 30 min and a further 30 min at ambient temperature, then it was provided with end fittings and attached to a HPLC pump. A mixture of ethyl vinyl ether in acetonitrile (40 wt.%) was pumped through the column to remove the initiator and any non-reacted monomers.

For the generation of the weak anion exchange functionality (Fig. 1), the monolith was treated with a mixture of equal volumes of diethyl amine, saturated aqueous Na_2CO_3 and 2-propanol. This mixture was pumped through the monolith at a flow rate of 0.5–3 mL/h at a temperature of 65 °C for 3 h. Then the monolith was sealed and left at the same temperature overnight. Finally, the column was cooled to room temperature and flushed with 20 mL of water, 20 mL of methanol, then again with water before conditioning with phosphate buffer (0.05 mol/L, pH 7).

2.5. Relative rates of polymerization of $NBE\text{-}CH_2Br$ and $(NBE\text{-}CH_2O)_3SiCH_3$

The degree of polymerization of the monomer and crosslinker was investigated by studying the copolymerization kinetics of $NBE\text{-}CH_2Br$ and $(NBE\text{-}CH_2O)_3SiCH_3$. Due to the high activity of the 1st-generation Grubbs benzylidene-type initiator [$RuCl_2(PCy_3)_2(CHPh)$], triphenylphosphine was added (3000 ppm) to the polymerization solution to slow down the rate of polymerization. Such an addition of triphenylphosphine allows for a kinetic investigation at room temperature because the apparent rate constants (k_p) for both $NBE\text{-}CH_2Br$ and $(NBECH_2O)_3SiCH_3$ are reduced to the same extent. This means that the ratio of these two rate constants remains unaffected. Twelve aliquots were withdrawn from the polymerization mixture in 1-min-intervals before the sample was completely polymerized. Each aliquot was added to a quantitative volume of ethyl vinyl ether/ACN (40:60 vol.%) to quench the

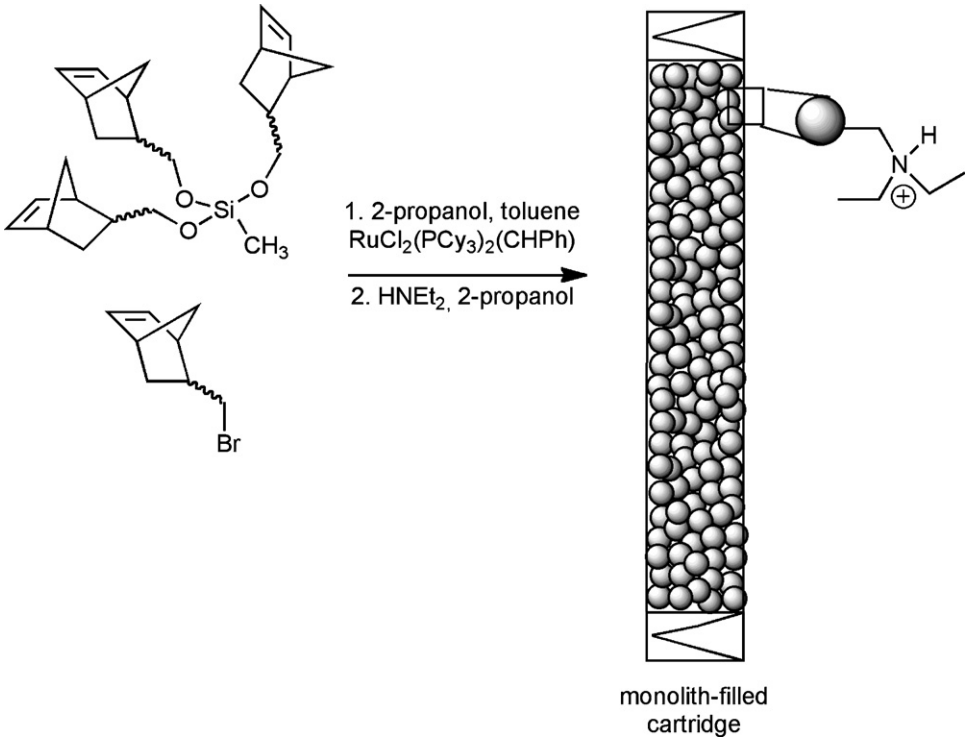

Fig. 1. Monolith synthesis and functionalization.

initiator, then filtered and subjected to GC–MS analysis to quantify the non-reacted monomer and crosslinker using dodecane as internal standard. Moreover, four monoliths were prepared under identical conditions. The amounts of non-reacted monomer and crosslinker were quantified as follows: each monolith was washed with 7 mL (~10 column volumes) of EVE/ACN (40:60 vol.%). The non-reacted amounts of monomers were quantified by analyzing these collects by GC–MS, applying calibration curves for the monomer and crosslinker standard solutions. These standard solutions were prepared in EVE/ACN (40:60 vol.%) solvent simulating the monolith collects.

3. Results and discussion

3.1. Degree of polymerization of NBE-CH_2Br and $(NBE\text{-}CH_2O)_3SiCH_3$

For monolith synthesis, norborn-2-ene (NBE) and $(NBE\text{-}CH_2O)_3SiCH_3$ were used as monomer and crosslinker, respectively. Earlier investigations [36] revealed that the use of this particular crosslinker results in a higher degree of crosslinking, which in turn translates into a better mechanical stability and higher permeability of the resulting monolith. In a first step, the apparent rate constants of polymerization for both norborn-2-ene-5-methylbromide ($NBE\text{-}CH_2Br$, k_{p1}) and the crosslinker ($(NBE\text{-}CH_2O)_3SiCH_3$, k_{p2}) were studied. In case $k_{p1}/k_{p2} \approx 1$, $NBE\text{-}CH_2Br$ must be expected to be evenly distributed *within* the structure-forming microglobules, However, in case $k_{p2}/k_{p1} > 1$, major parts of the $NBE\text{-}CH_2Br$ would be copolymerized at a comparably later stage of the copolymerization. In such case, $NBE\text{-}CH_2Br$ must be expected to become grafted mainly to the surface of the monolithic support and be thus accessible for further derivatization since the living chain termini are located at the phase border to 2-propanol [32,33]. In fact we found that the calculated apparent rate constant of polymerization of $(NBE\text{-}CH_2O)_3SiCH_3$ k_{p2} is about 5 times higher than the one of $NBE\text{-}CH_2Br$ (k_{p1}, Fig. S1). This strongly suggests that major parts of the $NBE\text{-}CH_2Br$ are in fact rather located at the migroglobule's surface than within these microglobules. Consequently, the weak anion exchange sites formed there from are mostly accessible by the analytes. This reduced copolymerization propensity of $NBE\text{-}CH_2Br$ with respect to the crosslinker thus offers an attractive alternative to surface modification of monoliths so far accomplished by surface grafting. Furthermore, the analysis of the effluents of the monoliths showed that ~50% of $NBE\text{-}CH_2Br$ and ~10% of $(NBE\text{-}CH_2O)_3SiCH_3$ remained non-reacted under the conditions applied for monolith preparation. This is another indication of the low polymerization propensity of the monomer compared to the crosslinker.

3.2. Determination of the ion-exchange capacity

The ion-exchange capacity of weak anion exchanger monolith was determined by elemental analysis thereby quantifying the nitrogen content of the monolith. The ion-exchange capacity determined that way was found to be 0.51 mmol/g-support. This means that ~80% of the monomer $NBE\text{-}CH_2Br$ reacted with diethyl amine. Furthermore, aiming on even higher anion exchange capacity, the amount of $NBE\text{-}CH_2Br$ located on the microglobule's surface might be further increased by increasing the polymerization time and/or polymerization temperature.

3.3. Monolith structure

Any in-depth analysis of newly introduced monoliths should be accomplished by an evaluation of the mechanical stability as well as by scanning electron microscopy (SEM) and inverse size exclusion chromatography (ISEC) [37,38]. The mechanical stability of a chromatographic support under operating conditions is best determined by measuring the pressure drop *vs.* flow rate for various common HPLC solvents. Here, water, methanol, tetrahydrofuran and acetonitrile were used applying flow rates up to 5 mL/min (≈16 mm/s, Fig. 2). In good accordance with Darcy's

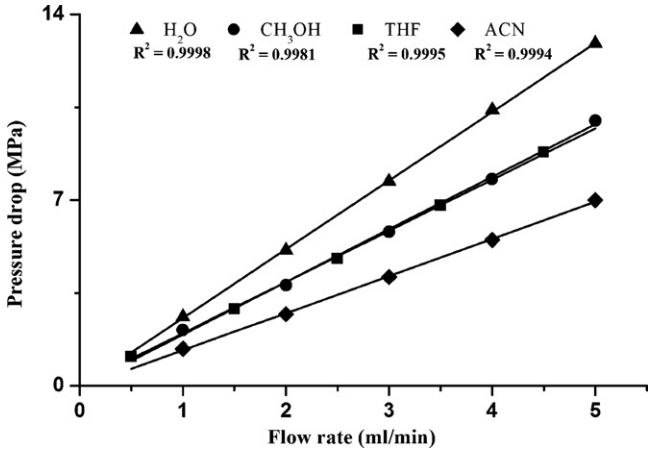

Fig. 2. Plot of pressure drop *vs.* flow rate for a ROMP-derived anion-exchange monolith (100 × 3 mm I.D.) applying water, methanol, tetrahydrofuran and acetonitrile as mobile phases.

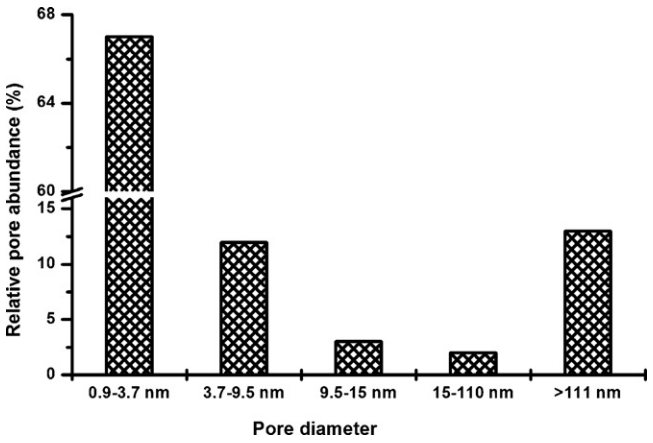

Fig. 4. Pore-size distribution of a ROMP-derived anion-exchange monolith (100 × 3 mm I.D.) determined by inverse size exclusion chromatography (ISEC) applying tetrahydrofuran as a mobile phase and polystyrene standards ($580 < M_n < 2{,}750{,}000$ g/mol).

law ($u = B_0 \cdot \Delta p/(\eta \cdot L)$); u = linear flow velocity; B_0 = column permeability; Δp = pressure drop; η = viscosity; L = column length), low pressure drops of 13, 10, 10 and 7 MPa were observed for water ($\eta = 1$ g/ms), methanol ($\eta = 0.6$ g/ms), tetrahydrofuran ($\eta = 0.49$ g/ms) and acetonitrile ($\eta = 0.36$ g/ms), respectively. This implies both good monolith permeability ($B_0 = 1.1 \times 10^{-11}$ m^2/s^2) for these solvents and an insignificant swelling of this material, particularly with THF. In fact, the finding that virtually no swelling of the polymeric support with THF was observed supports a high degree of crosslinking of the structure-forming microglobules, thereby confirming the presence of major amounts of NBE-CH_2Br at the surface of the microglobules. Moreover, the excellent line regression values (R^2) > 0.9981 for the four solvents applied, further confirm their high mechanical stability.

Next, we subjected the monoliths to scanning electron microscopy (SEM, Fig. 3). As can be seen, a microglobule diameter of 2–5 μm and a diameter of the flow channels in the range of ~10 μm were found. The latter explain for the good permeability of the polymeric support. Finally, the monolith was subjected to inverse size exclusion chromatography (ISEC) [37,38]. This method allows for determining the total porosity and pore size distribution, which both highly influence the column separation efficiency. Toluene and seven polystyrene samples of $580 < M_n < 2{,}750{,}000$ g/mol were studied by this method. The total volume fraction occupied by the mobile phase (ε_t) of the monolith studied was calculated to be 75%, thereby further explaining for the low back pressure encountered with various solvents. The volume fraction of pores within the globules (ε_p) and the volume fraction of the intermicroglobule void volume (ε_z) were found to be 15 and 60%, respectively. Fig. 4 illustrates the pore size distribution of this monolith dissected into various intervals ranging between 3.7 and 111 nm, covering the range of mesopores ($2 < \Theta < 50$ nm) and macropores ($\Theta > 50$ nm). It is quite clear that about 20% of all pores are located in the macropore range while the residual 80% are in the meso- and micropore range.

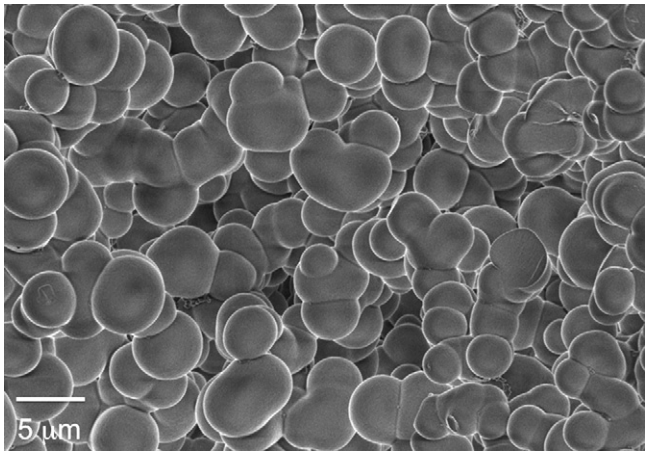

Fig. 3. SEM picture of a ROMP-derived anion-exchange monolith.

3.4. Separation of nucleic acids

3.4.1. Optimization of buffer mobile phase

Encouraged by the relatively high ion-exchange capacity of the monolith, a sample of *ds*-pUC 18 DNA Hae III digest consisting of eleven fragments (Table 1) was applied as sample. Applying a phosphate buffer (0.05 mol/L, pH 7) and NaCl as a gradient former, the eleven fragments were baseline separated within 12 min with the exception of a partial separation of fragments 7 and 8 (267 and 298 bp) (Fig. 5). The unique monolith separation efficiency is demonstrated and confirmed by the high resolution ranging from 2.51 to 18.73 as well the sharp peaks reflected by the small

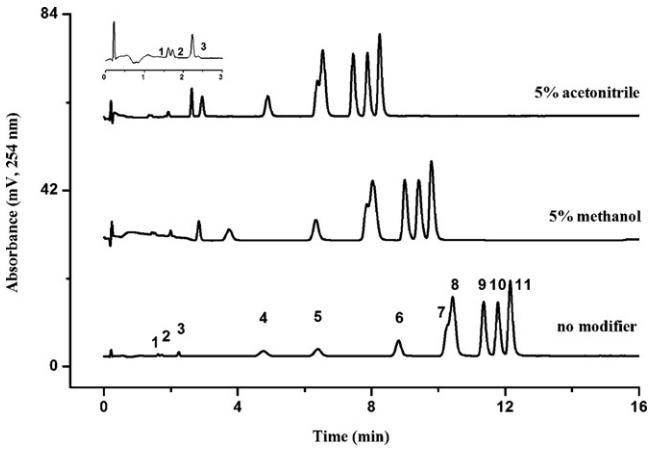

Fig. 5. Influence of organic modifiers as a mobile phase additive on the separation of *ds*-DNA fragments; mobile phase, A: phosphate buffer (0.05 mol/L, pH 7), B: A + NaCl (1.0 mol/L); gradient 0–60% B in 2 min; 60–85% B in 13 min; flow rate, 3.0 mL/min; $T = 50$ °C; sample, 3.0 μg of pUC18 DNA Hae III digest.

Table 2
Influence of organic modifiers as additives to the phosphate buffers on the speed and efficiency of separation of pUC 18 DNA Hae III digest by anion exchange applying a ROMP-derived anion-exchange monolith (100 × 3 mm I.D.) presented as retention times (t_R), peak width at peak half height ($\omega_{0.5}$) and resolution (R_s).

	No organic additives			5 vol.% methanol			5 vol.% acetonitrile		
Fragment	t_R (min)	$\omega_{0.5}$ (s)	R_s	t_R (min)	$\omega_{0.5}$ (s)	R_s	t_R (min)	$\omega_{0.5}$ (s)	R_s
1	1.691	2.5	1.20	1.492	2.0	1.10	1.482	1.5	1.10
2	1.757	2.4	6.92	1.621	1.9	6.43	1.542	1.4	10.71
3	2.259	2.8	18.73	2.004	2.3	11.00	1.95	1.2	15.42
4	4.759	6.7	10.68	2.835	3.0	6.67	2.623	1.8	4.33
5	6.821	7.0	10.48	3.743	6.6	14.28	2.935	3.3	16.29
6	8.81	6.5	7.64	6.335	6.2	10.24	4.9	5.3	11.65
7	–	–	–	7.858	4.3	1.06	6.384	3.7	1.21
8	10.426	8.5	4.74	8.033	7.4	5.38	6.542	5.5	6.56
9	11.351	5.3	2.89	8.995	5.2	2.81	7.452	4.3	3.55
10	11.78	5.2	2.51	9.41	5.2	2.54	7.88	4.2	3.02
11	12.148	5.2	–	9.787	5.2	–	8.244	4.3	–

peak widths at peak half height ($\omega_{0.5}$) ranging from 2.4 to 8.5 s (Table 2). Organic modifiers are usually applied in such a separation of nucleic acids to overcome the hydrophobic interaction between the nucleic acid nitrogenous bases with the monolith support. In attempt to optimize the mobile phase, methanol was added (5 vol.%) to the phosphate buffer. Applying the same chromatographic conditions, the total analysis time was shortened by almost 2.5 min and the $\omega_{0.5}$-values decreased by 20%, now ranging from 1.9 to 7.4 s, while maintaining the good resolution (Table 2). In addition, the separation of the two fragments 7 and 8 was slightly improved as two peak apexes could be observed, which was not the case in the absence of any organic modifier (Fig. 5). Switching from methanol to acetonitrile as organic modifier, the separation further improved significantly. The eleven fragments were separated in about 8 min accompanied by a decrease in $\omega_{0.5}$ to 1.2 and 5.5 s, while still maintaining good resolution (Table 2, Fig. 5).

3.4.2. Separation of ds-pBR322 DNA Hae III digest

The *ds*-pBR322 DNA Hae III digest consists of 22 fragments (Table 1); however, only 19 fragments of the 51–587 base pairs (bp) of *ds*-DNA have been separated by capillary electrophoresis [21,22], by ion-exchange [20] and by ion-pair reversed-phase HPLC on poly(styrene-*co*-divinylbenzene) supports [13,14]. The smallest four fragments in *ds*-pBR 322 consisting of 8, 11, 18, 21 bp can usually not be resolved by these methods. Moreover, fragments 123 and 124 bp are hard to separate under common separation conditions. This is a consequence of the wide range in fragment size (8–587 bp), which requires a lot of column and mobile phase optimization. For the new monolithic columns prepared, a two-step gradient in NaCl as a gradient former allowed for a very good separation of this sample (Fig. 6). 19 Fragments could be detected and baseline separated in a short time of about 12 min. However, co-elution of fragments 14 and 15 (213, 234 bp) and of 9, 20 and 21 (458, 504 and 540) was experienced under these conditions. It is worth noting that fragments 11 and 12 (123 and 124 bp) were almost separated and the 8, 11, 18 and 21 base pair fragments were clearly detected (Fig. 6), a task that has always been a challenge in both RP-HPLC and electrophoresis [13,14,22]. The monolith efficiency in separating *ds*-pBR322 DNA Hae III digest was comparable to that observed for the *ds*-pUC 18 DNA Hae III digest, which is evidenced by high resolution in the range of 1.4–11 and very small peak widths at peak half height ranging from 1.8 to 6.3 s for the 19 fragments separated (Table 3). Concerning monolith capacity, a totals amount of 3 μg was injected, thereby still enabling clear UV-detection of the small fragments containing 8–21 bp. Since the concentration of the single fragments in the *ds*-pUC 18 DNA Hae III and the *ds*-pBR322 DNA Hae III digest was unknown, both the limit of detection as well as the linearity range could not be assessed.

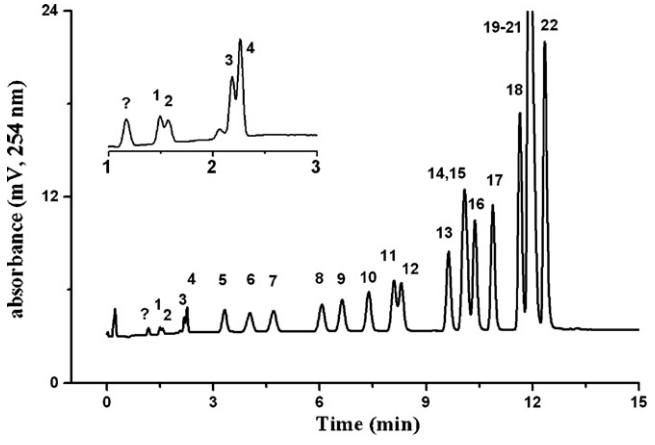

Fig. 6. Separation of pBR322 DNA Hae III digest fragments on a ROMP-derived anion-exchange monolith (100 × 3 mm I.D.); mobile phase, A: phosphate buffer (0.05 mol/L, pH 7) + 5% ACN, B: A + NaCl (1.0 mol/L); gradient 0–50% B in 2 min; 60–78% B in 13 min; flow rate, 3.0 mL/min; T = 50 °C; sample, 3.0 μg of pBR322 DNA Hae III digest.

Table 3
Separation efficiency for a pBR 322 DNA Hae III digest by anion exchange on a ROMP-derived anion-exchange monolith (100 × 3 mm I.D.) presented as retention times (t_R), peak width at peak half height ($\omega_{0.5}$) and resolution (R_s).

Fragment	t_R (min)	$\omega_{0.5}$ (s)	R_s
1	1.482	2.2	1.44
2	1.636	2.4	10.32
3	2.221	1.8	1.49
4	2.264	1.9	10.98
5	3.321	4.9	4.49
6	4.032	6.3	3.74
7	4.700	6.4	7.82
8	6.063	5.9	3.47
9	6.634	5.7	4.77
10	7.383	5.4	4.84
11	8.095	5.0	1.44
12	8.299	5.0	9.73
13	9.633	4.6	2.99
14	10.083	6.0	2.00
15	–	–	–
16	10.376	4.4	4.11
17	10.877	4.2	6.61
18	11.644	4.0	1.94
19	11.909	5.7	3.17
20	–	–	–
21	–	–	–
22	12.344	4.0	–

3.5. Run-to-run reproducibility

Five consecutive runs of pUC 18 DNA Hae III digest were performed under identical conditions as one run per working day. The monolith was conditioned for 1 h and two blank runs were performed prior to sample separation. Ideal run-to-run reproducibility was observed for the values of retention time, resolution, and peak width at peak half height with relative standard deviations (RSD, σ_{n-1}) < 0.2% throughout.

4. Conclusion

A ROMP-derived monolithic anion exchanger based on poly(NBE-CH_2Br-*co*-(NBE-CH_2O)$SiCH_3$) was prepared via reaction of the bromomethyl group with diethylamine. The different rates of polymerization for NBE-CH_2Br and (NBE-CH_2O)$SiCH_3$ allowed for realizing a highly crosslinked, pressure and mechanically stable, highly permeable monolithic matrix with poly(NBE-CH_2Br) chains mainly attached to the surface of the structure-forming microglobules of the polymeric monoliths thereby mimicking the previously *in situ* derivatization by a much simpler approach. The resulting monoliths demonstrated relatively high ion-exchange capacity and allowed for the fast and highly efficiency anion exchange separation of nucleic acids as double-stranded *ds*-DNA. Small peak widths at peak half height and good resolution were observed. The stability of the monoliths was demonstrated by low RSD values <0.2% in t_R, R_s and $\omega_{0.5}$.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.08.053.

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