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Microwave-immobilized polybutadiene stationary phase for reversed-phase high-performance liquid chromatography

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Abstract

Polybutadiene (PBD) has been immobilized on high-performance liquid chromatography (HPLC) silica by microwave radiation at various power levels (52–663 W) and actuation times (3–60 min). Columns prepared from these reversed-phase HPLC materials, as well as from similar non-irradiated materials, were tested with standard sample mixtures and characterized by elemental analysis (%C) and infrared spectroscopy. A microwave irradiation of 20 min at 663 W gives a layer of immobilized PBD that presented good performance. Longer irradiation times give thicker immobilized layers having less favorable chromatographic properties.

Keywords: Stationary phases, LC; Polybutadiene–silica stationary phases; Microwave-assisted immobilization

1. Introduction

Major advances have occurred over the last 10–20 years in the design and development of high-performance liquid chromatographic (HPLC) stationary phases. One focus has been on the elucidation of the pore and particle structure of HPLC packings with regard to better mass transfer kinetics, higher column performance and faster analysis. This led to the family of macroporous packings of mean pore diameter >50 nm and to non-porous packings [1]. Initially the most common chromatographic support used was unmodified porous silica, but today many modifications of the silica support have been investigated [2–6], as well as the use of other supports such as zirconia [7–16], titania [7,15–17] and alumina [15,16,18,19], although porous silica continues to be the most used for the preparation of reversed-phase HPLC materials. Another focus has been on novel concepts and improvements of the stationary phase chemistry of HPLC packings by means of several types of polymeric coatings on the support, such as polybutadiene (PBD) [8–14,19–23], polyethylene [24–26], polystyrene [27,28], poly(dimethylsiloxane) [29], poly(methyloctylsiloxane) [30–37] and poly(methyloctadecylsiloxane) [37,38], immobilized by several different processes, including thermal treatments [31,32,35,37], γ radiation [23,30–33,38] and microwave radiation [31,32].

The use of polybutadiene in the preparation of stationary phases for RP-HPLC was first studied with silica support particles [20–22] and later with zirconia particles [8–14], using dicumyl peroxide as immobilization initiator.

We have recently prepared a stationary phase consisting of PBD coated on porous silica particles immobilized by γ radiation, which showed good chromatographic behavior and stability [23]. In the present paper we extend our work using another immobilization procedure, microwave radiation, investigating the effect of different microwave power levels and irradiation times on the immobilization of PBD onto silica particles for use in RP-HPLC.

2. Experimental

2.1. Materials

Hexane (Mallinckrodt, HPLC-grade) was used as solvent in the preparation of the stationary phase. Filtered methanol (Mallinckrodt, HPLC-grade) and water (Milli-Q) were used to prepare the mobile phases. Filtered chloroform (Merck, analytical reagent grade) and methanol were used for the extraction of excess polymer. The compounds

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used for chromatographic testing (uracil, acetone, benzonitrile, benzene, toluene and naphthalene) were analytical reagent grade and were not further purified. The silica support used was Rainin (100-5) (Varian, PK.101-K5), having 5 μm spherical porous particles. The PBD polymer (mean molar mass of ~5000, 80% 1,4-*cis*/*trans* and 20% vinyl) was obtained from Aldrich.

2.2. Preparation of stationary phase

2.2.1. Preparation of PBD coatings

The stationary phase was prepared with a 50% loading of PBD (1 g PBD g^{-1} silica which had been dried at 150 °C for 24 h). To load the PBD into the silica pore system, a solution of PBD in hexane (12 ml for each 1 g of PBD) was mixed with a suspension of silica in hexane (12 ml for each 1 g of silica particles). The suspension was stirred for 3 h. After the hexane was slowly evaporated, without stirring, at room temperature and the stationary phase remained in storage at room temperature for 6 days.

2.2.2. Irradiation of stationary phase

For each experiment, 3 g of the prepared stationary phase were put into a polytetrafluorethylene (PTFE) container. A household microwave oven (Electrolux, ME27) with rotating plate and operated at a frequency of 2.45 GHz, was used in this immobilization process. Initially the microwave power was optimized for the fixed microwave irradiation time of 30 min. After that, the microwave power was fixed in 663 W and the microwave irradiation time was varied. During the experiment, after each 3 min of microwave irradiation, the container was withdrawn from the oven, cooled for 2 min and returned to the oven to continue the irradiation.

2.2.3. Extraction procedure

After immobilization of the stationary phases, tubes containing the irradiated phase were connected to a Waters 510 pump (Milford, MA, USA) for extraction of all the non-immobilized (excess) PBD. First, chloroform and then methanol were passed through the irradiated material at 1 ml min^{-1} for 3 h at ambient temperature, for each solvent. A portion of non-irradiated stationary phase was also submitted to the extraction procedure. After this procedure, the stationary phase contained in the tube was removed and the solvent was evaporated at room temperature.

2.3. Physical and chemical characterization of stationary phases

2.3.1. Percent carbon, surface area and mean pore size

Elemental analyses (%C and %H) were obtained from the packing materials prepared under different conditions. Duplicate determinations were made with a Model CHN-2400 Perkin-Elmer Analyzer. These data permitted the determination of the real immobilized PBD content in each prepared stationary phase. From these data, the specific mass of PBD, $\bar{m}_{PBD}$, which represents the mass of polymer per gram of silica, was calculated using the formula: $\bar{m}_{PBD} = \%C/(88.9 - \%C)$, since 88.9% of the PBD refers to carbon. With the specific mass data the polymer layer thickness, τ, was calculated, as follows:

$$\tau = -\frac{\sqrt{d^2 - Fd^2} - d}{2}$$

where F is the immobilized fraction:

$$F = \frac{\bar{m}_{PBDimm}}{\bar{m}_{PBDfullpores}}$$

and d the mean pore diameter. It is assumed for this calculation that the pores of the silica have a constant diameter and that the immobilized polymer is characterized by a layer of constant thickness, τ, on the pore walls [23]. The specific pore volume, v_p, for Rainin silica, is 0.55 ml g^{-1} of SiO_2 and the density of PBD is 0.89 g ml^{-1}. From this pore volume, the mass of polymer that fills the pore system is 0.49 g_{PBD} $g_{silica}{}^{-1}$.

Specific surface area (S_{BET}, N_2) determinations were carried out with a Model 2300 Micromeritics FlowSorb II instrument. The mean pore size of the silica particles was obtained with a Model 9320 Micromeritics Poresizer mercury intrusion porosimeter.

2.3.2. Infrared spectroscopy

Infrared spectra were obtained of the PBD, the silica and the stationary phases (immobilized by microwave radiation and non-irradiated) using a BOMEM model FT-IR DA-8 instrument.

2.4. Chromatographic evaluation of the stationary phase

2.4.1. Column packing

Columns (60 mm × 3.6 mm i.d.) were made from type 316 stainless-steel tubing. The internal surface was polished using a technique developed in our laboratory [39]. The columns were downward packed using 10% (w/v) slurries of each stationary phase in chloroform. A constant packing pressure of 34.5 MPa (Haskel packing pump) was used, with methanol as propulsion solvent. Columns were conditioned for 2 h with a mobile phase consisting of methanol–water 70:30 (v/v) at 0.3 ml min^{-1}. Each phase was evaluated chromatographically with duplicate columns.

2.4.2. Chromatographic evaluation

The chromatographic evaluation of column performance was done with a modular HPLC system equipped with a Shimadzu LC-10 AD pump, a Rheodyne Model 8125 injection valve (5 μl loop) and a Shimadzu Model SPD-10 AV UV-Vis detector (at 254 nm). Data acquisition used Chrom Perfect for Windows, version 3.52 and Report Write Plus software (Justice Innovations).

The standard sample mixture used in this study contained the solutes: uracil, acetone, benzonitrile, benzene, toluene and naphthalene. The mobile phase was methanol–water (70:30, v/v) at 0.2 ml min^{-1} for all the stationary phases. The optimal flow-rate was determined by a Van Deemter plot. The column dead time, t_M, was determined using uracil as an unretained compound. Chromatographic performance was evaluated by means of efficiency (plates per meter (N/L)), retention factor (k), resolution (R_s) and asymmetry factor (A_s) measured at 10% of the peak height.

3. Results and discussion

3.1. Microwave oven calibration and investigation of microwave patterns

In order to establish the best microwave immobilization conditions, a calibration of the microwave oven using a US Environmental Protection Agency (EPA) method [40] was carried out. The maximum power achieved was 663 W. Thermal paper was used to locate the position of maximum microwave intensity (near the edge of the plate). All sample irradiations were made at this location.

3.2. Characterization of the stationary phases

The chromatographic parameters of columns obtained using stationary phases immobilized by 30 min irradiation at different microwave power levels are shown in Table 1. The stationary phase immobilized at maximum microwave power produced columns that exhibited better efficiency values than were obtained at lower microwave power levels. The percent carbon data obtained for these phases show that the amount of PBD immobilized on the silica increases with the increase in microwave power level. This greater amount of immobilized PBD is associated with a greater polymer-layer thickness, which results in improved chromatographic properties compared to those from non-irradiated stationary phases.

Table 1
Chromatographic parameters of PBD stationary phases non-irradiated and irradiated by 30 min microwave irradiations at several microwave power levels

Power (W)	Chromatographic parameters[a]				Carbon (%)	Layer thickness (τ) (nm)
	N/L[b]	A_s[b]	k[b,c]	R_s[d]		
0	58400 ± 1300	1.7	1.5	3.5	5.7	0.4
52	52400 ± 1200	1.7	1.4	3.4	5.6	0.4
195	73100 ± 300	1.4	2.1	4.9	8.0	0.6
596	78900 ± 1800	1.1	2.2	5.2	8.3	0.6
663	93800 ± 2700	1.1	5.0	7.5	14.4	1.3

[a] Chromatographic conditions: mobile phase, methanol–water (70:30, v/v); flow-rate, 0.2 ml min^{-1}; volume of injected sample: 5 μl and detector, UV at 254 nm. Values shown averages of chromatograms with two different columns.
[b] Calculated for the naphthalene peak.
[c] Column dead time was measured with uracil.
[d] Calculated for the toluene–naphthalene pair.

Table 2
Chromatographic parameters of the stationary phases prepared with PBD on silica supports irradiated by microwave radiation at 663 W power level for various irradiation times

Time (min)	Chromatographic parameters[a]				Carbon (%)	Layer thickness (τ) (nm)
	N/L[b]	A_s[b]	k[b,c]	R_s[d]		
3	78700 ± 1500	1.3	2.4	5.3	8.8	0.7
10	82500 ± 500	1.6	3.7	6.4	11.7	1.0
20	96700 ± 900	1.1	3.9	7.0	12.4	1.0
30	93800 ± 2700	1.1	5.0	7.5	14.4	1.3
40	93900 ± 700	1.2	5.3	7.7	15.1	1.4
50	73800 ± 800	1.6	7.3	7.4	18.3	1.8
60	71200 ± 2400	1.4	7.8	7.6	17.8	1.7

[a] Chromatographic conditions: mobile phase, methanol–water (70:30, v/v); flow-rate, 0.2 ml min^{-1}; volume of injected sample: 5 μl and detector, UV at 254 nm. Values shown averages of chromatograms with two different columns.
[b] Calculated for the naphthalene peak.
[c] Column dead time was measured with uracil.
[d] Calculated for the toluene–naphthalene pair.

The improvement in chromatographic properties does not continue to increase, however. The optimal microwave irradiation time at maximum power (663 W) was 20 min, as seen in Table 2. The chromatographic efficiency values increase up to 20 min. After this time, they begin to decrease, although the percent carbon continues to increase. The different values for retention factor (k) show that the columns present different separation characteristics as a function of the degree of polymer loading promoted by different immobilization times. In the best condition, the thickness of the PBD layer on the porous silica was 1.0 nm. This layer thickness formed is the same as that obtained when PBD was immobilized onto silica by γ radiation [23]. That study showed that a very small absorbed dose of 5 kGy or less causes the radiation immobilization to arrive at a pseudo-plateau where the thickness value is well defined at ∼1.15 ± 0.05 nm. Thus, it appears that some sort of well-defined immobilized PBD monolayer of this thickness is involved, which permits easier diffusion of solutes into and out of the pore system. The decrease in efficiency of the immobilized phases irradiated for longer times may be result of the partial blockage of smaller pores by polymer. A typical chromatogram using optimized conditions is shown in Fig. 1 where we can see that the column was efficient for the separation of the test mixture, exhibiting good chromatographic performance.

The presence of immobilized PBD on the silica pore surfaces can be confirmed in Table 3. The selective blockage of small pores of the silica results in an increase of mean pore size as well as decreases in surface area and specific pore volume.

Fig. 2 shows infrared spectra of the PBD and silica. The region characteristic of the PBD indicates the presence of *cis*-1,4, *trans*-1,4 and vinyl structures that give rise to

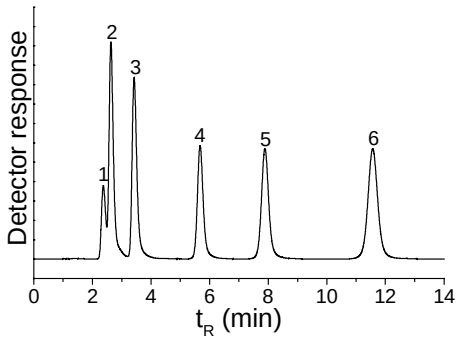

Fig. 1. Chromatographic behavior of a stationary phase irradiated by microwave radiation with a power of 663 W for 20 min. Test mixture: (1) uracil; (2) acetone; (3) benzonitrile; (4) benzene; (5) toluene and (6) naphthalene. Chromatographic conditions: mobile phase, methanol–water (70:30, v/v); flow-rate, 0.2 ml min^{-1}; volume of injected sample, 5 μl and detector, UV at 254 nm.

Table 3
Mean pore size, specific pore volume and specific surface area (S_{BET}) of silica and stationary phase non-irradiated and irradiated by microwave radiation at power 663 W and 20 min microwave irradiation time

Type of material	Mean pore size (nm)	Specific pore volume (ml g^{-1})	S_{BET} (m^2 g^{-1})
Silica	11.4	0.55	188
Stationary phase non-irradiated	11.8	0.46	157
Stationary phase irradiated by microwave radiation	13.8	0.27	78

sharp bands at 728, 966 and 911 cm^{-1}, respectively [41]. The region characteristic of the silica, near 3400 cm^{-1} is due to vibrations of the hydroxyl groups having a hydrogen bridge to physically adsorbed water. Another band, around 1100 cm^{-1}, is attributed to the siloxane groups

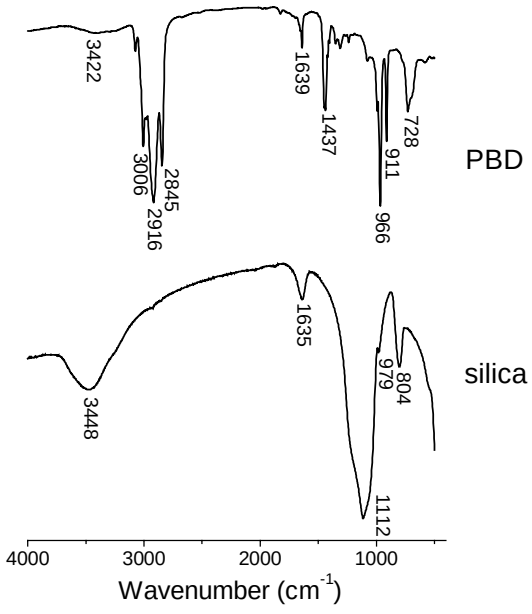

Fig. 2. Infrared spectra of PBD and silica Rainin.

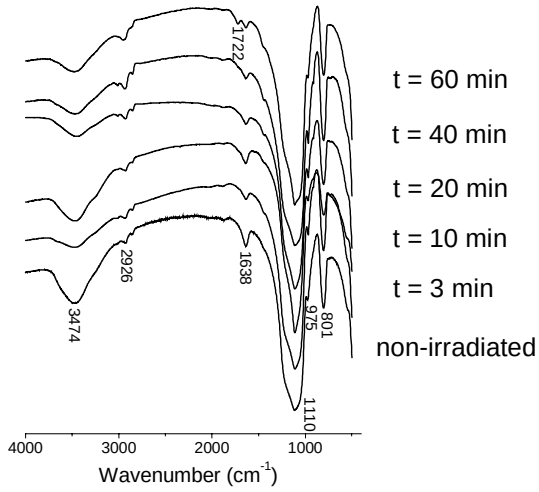

Fig. 3. Infrared spectra of PBD stationary phases non-irradiated and irradiated by microwave radiation during different microwave irradiation times.

and that at 979 cm^{-1} is assigned to the stretching vibration of free silanols [42]. The infrared spectra of the microwave-immobilized stationary phases, irradiated for different times and non-irradiated (Fig. 3), show bands that are characteristic of PBD and silica. For the 60 min microwave irradiation time, the infrared spectrum shows a band at 1722 cm^{-1}, which does not appear in the spectra related to the other irradiation times. This band is characteristic of ketone formation [43,44], indicating that some oxidation of the PBD has taken place. This oxidation probably occurred as a result of the many times that the stationary phase was withdrawn from the oven to permit thermal cooling.

4. Conclusions

Microwave radiation can be used to immobilize PBD on the surfaces of porous silica. The best results were obtained with the maximum microwave power (663 W) and 20 min of irradiation time, producing a 1.0 nm layer of PBD on the surface of HPLC porous silica. Columns packed with this phase have good separation efficiencies for the solutes studied.

Long irradiation times (>50 min) tend to give high levels of PBD oxidation, associated with a thick layer (>1.7 nm) which gives lower separation efficiencies.

The use of microwave radiation in the preparation of stationary phases appears to be an attractive alternative to other immobilization procedures in that chemical activators are not required, the procedure is fast and simple and the cost of the microwave irradiation is low.

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