Radiopaque iodinated ethers of poly(vinyl iodobenzyl ether)s: Synthesis and evaluation for endovascular embolization

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ABSTRACT: Leachable-free radiopaque iodinated polymers were designed as long-lived embolization materials visible by X-ray tomography. This is a definite improvement over liquid embolics incorporating either radiopaque inorganic particles or iodinated polymers having hydrolysable ester bonds. Grafting 4-iodobenzyl or 2,3,5-triiodobenzyl groups to poly(vinyl alcohol) (PVAL) yields iodobenzyl ethers of PVAL having iodine contents in the range 40–70 wt %. Their solubility in solvents accepted for medical devices (DMSO and NMP), viscosity of concentrated solutions, precipitation behavior, radiopacity, and stability with respect to sterilization and hydrolysis were assessed. The solvent NMP allows the preparation of concentrated solutions of suitable viscosity for their application as liquid embolics. Precipitation in water yields a cohesive mass of material that can plug vascular malformations. A rationale to the properties is given in terms of the Hansen contributions to the Hildebrand solubility parameters. Iodobenzyl ethers of PVA resist hydrolysis whereas their corresponding iodobenzoyl esters leach iodinated fragments. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41791.

INTRODUCTION

Polymers exhibiting radiopaque properties allow the manufacture of materials for medical devices or products that can be noninvasively monitored *in vivo* using various X-ray imaging techniques, radiography, and tomography. Their various applications in medicine, dentistry, cosmetic surgery, and pharmacy include prostheses, catheters, surgical tools, stents, implants, inserts, embolization agents, dental products, capsules for gastrointestinal investigations to systems for local and/or controlled delivery of drugs and cells. One important medical application of radiopaque materials is image-guided treatment of vascular lesions by embolization, mostly aneurysms and arteriovenous malformations, using *in situ* implant-forming liquid formulations.[1,2] Positioning of the radiopaque embolic material using a catheter is monitored by the physician by means of the minimally invasive radiography.

Polymers can be made radiopaque using a variety of techniques.[3–5] Early technologies relied on blending polymers and either heavy metals or heavy metal salts as radiopacifying agents. The major drawback was the easy release of the radio-

paque agent from the inhomogeneous mixtures, resulting in radiopacity spreading throughout the body whereas the radiopaque marker was expected to label the location of the local administration. A first improvement consisted in cation-chelating polymers that allowed keeping the products homogeneous as a single-phase solution of radiopaque polymer containing heavy metal species. However, the radiocontrast agent still tended to leach into the body since it was not covalently bound to the polymer. A covalent linkage of the radiopaque moiety to the polymer is required in order to avoid radiological artifacts coming from the radiopaque leachables. The homogeneous, nonleaching radiopaque materials retain mechanical properties essentially equivalent to the parent polymer.

Owing to the high atomic number of iodine, iodinated polymers are suitable for their application as radiopaque materials. Basically, two synthetic routes can be used to prepare intrinsically radiopaque materials from iodinated polymers: (i) polymerization of radiopaque monomers (possibly combined with nonradiopaque co-monomers) and (ii) grafting preformed polymers with a radiopacifying moiety. Several materials synthesized through homo- or copolymerization of radiopaque monomers

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or oligomers have been reported: various polymethacrylates,[6–11] iodinated poly(ethylene glycol) acrylates,[12,13] poly(anhydride esters),[14] and polyurethanes.[15,16] Recent work also dealt with derivatization of preformed polymers: iodinated cellulose mixed esters,[17,18] polyurethanes,[19,20] natural rubber,[21,22] poly(methyl methacrylate-co-glycidyl methacrylate),[23–25] esters of poly(vinyl alcohol),[26–28] polyphosphazenes,[29] and poly(allylamine).[30]

Early studies on liquid embolics[31–34] focused on common water-insoluble polymers dissolved in water-miscible organic vehicles (mostly dimethylsulfoxide, DMSO) and added with an insoluble contrast agent.[35] Such liquid embolics, once injected into the body, lead to *in situ* precipitation of the polymer when its organic solvent rapidly mixes into the aqueous environment. However, entrapment of the contrast agent (e.g. bismuth trioxide, tantalum) inside the *in situ* formed polymer structure is not ensured and several troubles could occur such as sedimentation during catheterization, or slow release of the radiopaque materials to the surrounding areas. It is representing a major drawback for clinical follow-up and it could lead to side-effects related to the possible toxicity of leachable. A major progress for the liquid embolics forming an implant *in situ* was the changeover to intrinsically radiopaque polymers. Thus, Mottu *et al.*[17,36] synthesized and used iodine-containing cellulose mixed ethers solubilized in a wide variety of organic solvents for direct embolization of aneurysms and arteriovenous malformations. At the same time, Maurer *et al.*[15] investigated a novel radiopaque polyurethane dissolved in ethanol added with DMSO for hepatic artery embolization, but the polymer was slowly biodegradable, a property not desirable in our approach. A second set of experiments was carried out using an iodine-containing poly(vinyl alcohol) (I-PVAL) dissolved in DMSO or *N*-methylpyrrolidone (NMP) for treating aneurysms in pigs.[26–28] The iodinated poly(vinyl alcohol) used was a 4-iodobenzoyl ester of PVAL, exhibiting as such partial degradation.[27] Degradation of the iodinated polymer causes major troubles. In particular, leaching of iodinated materials and diffusion to the surroundings can lead to malfunction of the medical device.

The aim of the present study was thus to develop biostable iodinated polymers for vascular embolization purpose and control by X-ray fluoroscopy or tomography. Because of the known chemical stability of the ether linkage, iodinated ethers of PVAL were synthesized, a type of compound not yet described in the literature. The compounds selected were mono- and triiodobenzyl ethers of PVAL (MIB-PVAL, respectively TIB-PVAL), namely poly(vinyl 4-iodobenzyl ether) and poly(vinyl 2,3,5-triiodobenzyl ether). Homo- or copolymerization of the corresponding iodinated monomers was not retained as only low molecular weight materials could be generally prepared due to the bulky nature of the iodinated aromatic moieties.[6,9,37] Such a feature does not provide precipitation behavior suitable for embolization purpose of the polymers from the organic solutions.[36] Our compounds were prepared by the Williamson etherification reaction of PVAL with mono- and triiodobenzyl bromide. Three grades of PVAL of molar masses 13, 47, and 61 kDa were used as the molar mass of the iodinated polymer has a strong influence on the viscosity and injectability of the solution used for embolization, the precipitation behavior and the aspect of the solid plug formed.[35] The synthesis and the chemical characterization of these materials are reported. Their solutions in DMSO or NMP were examined for their ability to form, upon contact with an aqueous environment, a coherent mass of material by precipitation that could embolize cerebral vascular disorders. Viscosity and radiopacity of the organic solutions were determined in comparison with the Onyx® Liquid Embolic System approved for the treatment of brain arteriovenous malformations and aneurysms, consisting of DMSO solutions of ethylene-vinyl alcohol copolymer added with micronized tantalum powder for radiographic visualization. An *in vitro* model of aneurysms previously developed[38] was used for the assessment of the embolization properties of the iodinated polymers. Finally, the chemical stability of the iodobenzyl ethers of PVAL was assessed by comparison with the corresponding iodinated esters of PVAL, namely poly(vinyl 2,3,5-triiodobenzoate) and poly(vinyl 4-iodobenzoate). For this purpose, nanoparticles with large surface area were prepared by the nanoprecipitation method.[39,40]

MATERIALS AND METHODS

Starting Reagents

Poly(vinyl alcohol)s, Mowiol® 6–98 ($M_w = 47,000$ Da), Mowiol® 10–98 ($M_w = 61,000$ Da), and PVAL 87–89% hydrolyzed ($M_w = 13,000$ Da) were supplied by Sigma-Aldrich; 4-iodobenzyl bromide was supplied by AmplaChem (Carmel, IN 46032); 4-iodobenzoyl chloride (97%) was purchased from Sigma-Aldrich; 2,3,5-triiodobenzoic acid and 2,3,5-triiodobenzoyl chloride were purchased from Changzhou Ilhang Fine Chemical Co. Ltd (China). Pluronic® F68 was supplied by Molekula (Gillingham, UK). Solvents and other reagents used in this study were purchased from Acros Organics or Sigma-Aldrich. The water-miscible organic solvents used to prepare embolic liquids were *N*-methylpyrrolidone (NMP) and dimethylsulfoxide (DMSO) both purchased from Acros Organics, and Onyx® 18, 34, and 500 was supplied by eV3 (Bonn, Germany).

Synthesis of Intermediate Compounds

2,3,5-Triiodobenzyl Alcohol (1). A solution of BH_3-tetrahydrofuran (1*M*, 0.65 mol, 7 eq) was added drop-wise to a solution of 2,3,5-triiodobenzoic acid (0.09 mol, 1 eq) in dry tetrahydrofuran (122 mL) at 0°C under dry nitrogen gas flow.[41] The resulting mixture was stirred 1 h 15 min at 0°C and 1 h at room temperature. At the end of the reaction, a white solid has precipitated and a cold mixture of tetrahydrofuran/water (13/2) was slowly added, followed by a saturated cold aqueous solution of sodium bicarbonate. A white precipitate appeared after stirring for 1 h. The solid was recovered by filtration and washed with water and cold absolute ethanol.

2,3,5-Triiodobenzyl Bromide (2). A solution of phosphorous tribromide in dry THF (0.088 mol, 2 eq) was added to a solution of 2,3,5-triiodobenzyl alcohol (0.044 mol, 1 eq) in dry THF at 0°C under dry nitrogen gas flow. The reaction mixture was stirred for 5 min at 0°C and 20 min at room temperature, and then a cold mixture of water/dichloromethane (1/1) was added. The resulting aqueous phase was extracted with dichloromethane. The combined organic extracts were subsequently washed with an aqueous solution of sodium hydrogen carbonate, with pure water, dried over Na_2SO_4, filtered and
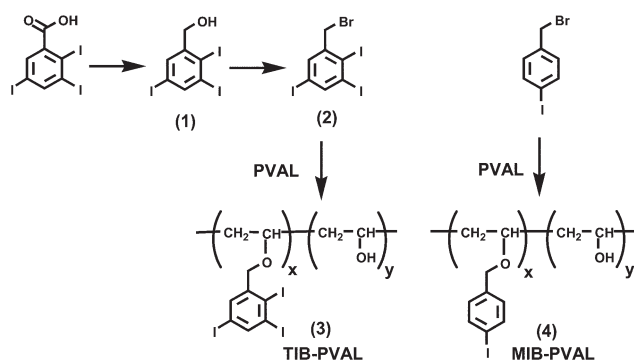
Figure 1. Synthesis pathway of the iodobenzyl ethers of PVAL.

concentrated. The white solid which has crystallized was filtered and washed with cold methanol.[42]

Synthesis of Iodobenzyl Ethers of PVAL
The general synthesis scheme of iodinated poly(vinyl alcohol)s by means of Williamson etherification is presented in Figure 1. The synthesis procedure was the same for the three PVAL grades of different molar masses ($M_w = 13$ kDa, 47 kDa, and 61 kDa).

Poly(vinyl 2,3,5-triiodobenzyl ether) (TIB-PVAL) (3). PVAL (9 mmol, 1 eq) was dissolved in anhydrous NMP (30 mL) by stirring at 130°C under nitrogen gas flow until the polymer dissolved. The PVAL solution was cooled down to 50°C and ground and dried sodium hydroxide powder (18 mmol, 2 eq) was added, followed by 2,3,5-triiodobenzyl bromide (9 mmol, 1 eq). Stirring was continued for 5 h; the mixture was cooled to room temperature and cold water (30 mL) was added under stirring for precipitation of the polymer. After filtration, the precipitated solid was recovered and washed with methanol. The crude solid was analyzed by ^{1}H NMR. It contained 51 mol % of grafted PVAL and 49 mol % of starting reagents and side products. Since those impurities are small molecules, they appeared in the NMR spectra as sharp lines popping up from the broad lines of the grafted polymer. This was clearly visible in the aromatic region.

For the purification, several steps of precipitation were necessary. The number of precipitation steps was decided from ^{1}H NMR analyses of the polymer. A supplementary precipitation step was applied till the sharp lines corresponding to the nongrafted materials had disappeared from the spectrum. The same procedure was applied for TIB-PVAL 13 kDa and 47 kDa: first, the crude solid was dissolved in DMSO/NMP (1/1 v/v) mixture ($C = 15$ wt %) and same volume of methanol was added; a pasty material precipitated and was recovered. The purity was not satisfactory after one precipitation process. A second similar purification step was made but using a solution in pure NMP ($C = 30$ wt %) and this step was repeated until the grafted polymer was pure. Grafted polymer was obtained as a beige solid with an overall yield of 19%.

In case of TIB-PVAL 61 kDa, the first precipitation step was made using a solution in NMP ($C = 35$ wt %) and same volume of methanol was added. Then, two precipitations from a NMP solution at 40 wt % followed by two precipitations from

a NMP solution at 45 wt % yielded the grafted polymer free from starting reagent and side product (yield = 25%).

Poly(vinyl 4-iodobenzyl ether) (MIB-PVAL) (4). PVAL (12 mmol, 1 eq) was dissolved in anhydrous NMP (40 mL) by stirring at 130°C under nitrogen gas flow until the polymer dissolved. The reaction mixture was then cooled down to 50°C and 4-iodobenzyl bromide (12 mmol, 1 eq) was added, followed by ground and dried sodium hydroxide (24 mmol, 2 eq). Stirring was continued for 4 h, the mixture was cooled to room temperature and 40 mL of cold water was added under stirring for precipitation of the polymer. After filtration, a precipitated solid was recovered and washed with methanol. Analysis by ^{1}H NMR showed that the crude solid contained 56 mol % of grafted PVAL and 44 mol % of starting reagents and side products.

Purification method was identical for the polymers of the three different molar masses: first, the crude solid was dissolved in NMP ($C = 30$ wt %) and four volumes of cold methanol were added, causing the precipitation of a pasty material. A second step consisted in the dissolution of the solid in NMP ($C = 40$ wt %) followed by addition of five volumes of methanol. The grafted polymer was obtained free from impurities with an overall yield of 52%.

Synthesis of Iodobenzoyl Esters of PVAL
Poly(vinyl 4-iodobenzoate) (5) and poly(vinyl 2,3,5-triiodobenzoate) (6): PVAL (19 mmol, 1 eq) was dissolved in dry NMP (30 mL) by stirring for 5 min at 130°C under nitrogen gas flow till the polymer was dissolved. The solution was cooled down to 0°C by immersing the round bottom flask into an ice bath. A solution of 4-iodobenzoyl chloride or 2,3,5-triiodobenzoyl chloride (19 mmol, 1 eq) in NMP (6 mL) was added dropwise; it was subsequently followed by additions of dry pyridine (23 mmol, 1.2 eq) and 4-dimethylaminopyridine (2.3 mmol, 0.12 eq). The temperature was increased to room temperature and the mixture was stirred for 6 h for poly(vinyl 4-iodobenzoate) and 12 h for poly(vinyl 2,3,5-triiodobenzoate). Then, the reaction medium was poured into a 100 mL beaker and cold water was added. A pasty material has precipitated, was filtered and washed with methanol.

The purification consisted in precipitation in an excess of poor solvent. For poly(vinyl 4-iodobenzoate), the crude pasty material was dissolved in NMP (concentration: 14 wt %) and 100 mL of an aqueous solution of sodium hydrogen carbonate was added upon stirring. Pure grafted PVA precipitated, it was filtered and washed with methanol. In case of poly(vinyl 2,3,5-triiodobenzoate), the crude pasty material was dissolved in 40 mL of NMP ($C = 22$ wt %) and 150 mL of cold ethanol was added while stirring. A pasty material has precipitated. After removing the water and washing with methanol, pure grafted PVAL was obtained.

^{1}H NMR (300 MHz, DMSO-$d6$, δ) of poly(vinyl 4-iodobenzoate) (5): 1.05–2.4 (m, CH$_2$ PVAL chain, $2(x + y)$), 3.81 (s, CH$_b$ PVAL chain, y), 4.21–4.67 (m, OH), 5.37 (s, CH$_a$ PVAL chain, x), 7.10–7.90 (m, H aromatic, $4x$).

IR (cm^{-1}) of poly(vinyl 4-iodobenzoate) (5): 3390 (broad band of associated O—H), 2915 (C—H), 1708 (C=O of ester), 1664

(C—H aromatic), 1584 (C—H aromatic), 1391 (C—OH), 1261 (aromatic ester), 1096 (aromatic ester), 1005 (C—H aromatic), 748 (C—H aromatic).

^{1}H NMR (300 MHz, DMSO-d6, δ) of poly(vinyl 2,3,5-triiodo-benzoate) (6): 1.35-1.95 (m, CH$_2$ PVAL chain, 2(x + y)), 3.81 (s, CH$_b$ PVAL chain, y), 4.21–4.67 (m, OH), 5.37 (s, CH$_a$ PVAL chain, x), 7.71 (s, H aromatic, x), 8.34 (s, H aromatic, x).

IR (cm^{-1}) of poly(vinyl 2,3,5-triiodobenzoate) (6): 3360 (broad band of associated O—H), 2914 (C—H), 1712 (C=O of ester), 1649 (C—H aromatic), 1519 (C—H aromatic), 1364 (C—OH), 1260 (aromatic ester), 1181 (C—H aromatic), 1098 (aromatic ester), 1000 (C—H aromatic).

Characterization of the Iodinated Polymers

^{1}H NMR Analysis. The grafting reactions of PVAL with iodinated benzyl bromides and iodinated benzoyl chlorides were followed by ^{1}H NMR. Spectra of the iodinated PVAL solutions in deuterated dimethylsulfoxide (DMSO-d6) were recorded on Bruker ALS300 and DRX300 Fourier transform spectrometers. Processing was done using Bruker WINNMR-1D software package. The degree of substitution, DS = $x/(x + y)$, was calculated for each product by comparing the area under the peaks corresponding to the aromatic protons to the area under the peaks corresponding to the CH$_2$ of the PVAL backbone at $\delta = 1.3–2.0$ ppm.

IR Spectroscopy. FT-IR spectra were recorded in ATR mode using a Thermo Scientific iS50 FT-IR spectrometer equipped with an iS50 ATR module. A small amount of pure compound finely ground as dry powder was deposited on the diamond crystal of the ATR equipment, and the spectra were recorded between 400 and 4000cm^{-1} by accumulating 32 scans at 2 cm^{-1} resolution.

Elemental Analysis for Iodine Content. Elemental analysis of iodine in the polymer was performed by the Laboratorium für Organische Chemie/Mikrolabor at ETH-Zurich. The mass fraction of iodine can be calculated from the DS found using the NMR analyses and was compared with that measured by elemental analysis.

Thermogravimetric Analysis. A TGA instrument Netzsch TG 209 F1 was used for recording the mass loss of the samples held in an alumina crucible under dry nitrogen flow between 20°C and 900°C at 10°C min^{-1} heating rate.

UV Absorption. UV absorbance of solutions was measured in Hellma quartz cells of 1 mm thickness using a Varian Cary 50 spectrometer. The absorbance of the pure solvent was subtracted as a background.

Precipitation Behavior

The precipitation of the polymer was assessed by pouring a 20 wt % solution of polymer in the selected solvent into a large amount of water through a syringe fitted with a needle 21G (0.8 mm internal diameter). A large coherent mass of precipitate should be formed for embolization purpose upon exchange of the solvent with surrounding water. The type of precipitation was evaluated as hard, soft or flaky mass, where hard means a relatively rigid polymer shell, which ruptures when deformed by 20%, whereas soft describes a deformable, plastic polymer mass.

Flakes are suspended polymer aggregates, without formation of a cohesive polymer mass.

Viscosity Measurements

The viscosity is an important parameter for the choice of concentration in organic solution for the embolization. Viscosity measurements were performed at 25°C with a rheometer Bohlin and Haake CV0120 equipped with measurement set up of the cone-plate geometry (1/40 mm). The gap size was 30 μm. Mean viscosity from fit of Newtonian behavior over 1 to 1000 s^{-1} shear rate range is reported. Comparative measurements with commercial Onyx® 18, 34, and 500 were performed.

Radiopacity Evaluation

Solutions of the MIB-PVAL 13 kDa and TIB-PVAL 13 kDa at a concentration of 33 wt % in NMP were poured in radiolucent 1 mL Eppendorf vials. X-ray absorption was measured on a computerized tomograph scan (CT-scan, Skyscan 1076, Skyscan, Belgium) using a 0.5 mm aluminum window, under 50 kV and 200 μA. 180 degrees tomograms were acquired and reconstructed (Nrecon 1.5.1.4, Skyscan, Belgium); pixel grey level was averaged over the whole embolic image (ImageJ program, NIH). Calibration into Hounsfield units (HU) was made using water (0 HU) and air (−1000 HU) as standards.

In Vitro Embolization

Injectable embolizing compositions of iodinated polymers and Onyx® 34 commercial composition were tested for their ability to fill an *in vitro* aneurysm model. A 10 mm diameter sphere model, affixed to a glass tube, was used.[43,44] The model was flushed with saline using a rotary pump under a 30 cm s^{-1} flow speed mimicking blood flow. The embolizing composition was injected into the aneurysm model with a 21 G needle at a slow rate of 1 mL min^{-1}.

Assessment of Stability

Preparation of Nanoparticles. Nanoparticles of the ethers and esters of PVAL were prepared by nanoprecipitation method. Iodinated polymer was dissolved in THF (10 mL) at room temperature; the solution was then added under magnetic stirring by means of a syringe into the dispersing phase made of PBS buffer (20 mL) containing Pluronic® F68 surfactant. The aqueous phase turned milky as the organic phase was poured and a homogeneous milky dispersion was obtained at the end. THF was removed under reduced pressure, yielding an aqueous suspension of iodinated polymer nanoparticles. Concentrations of iodinated polymers and Pluronic® F68 were different according to the polymers used in order to obtain particles with an average diameter of 200 nm in all instances.

Nanoparticle Size Measurements. The particles size distribution was determined by dynamic light scattering (DLS) using a NanoZS instrument (Malvern, UK). The aqueous suspensions were diluted with deionized water before measurements so as the count rate was of the order 200 kHz. Measurements were performed at 25°C in triplicate. Data analysis using the method of cumulants (ISO13321) gave the z-average diameter and polydispersity index (*PdI*).

Stability with Respect to Hydrolysis. Suspensions of iodinated polymer nanoparticles in PBS pH 7.4 were incubated at 37°C
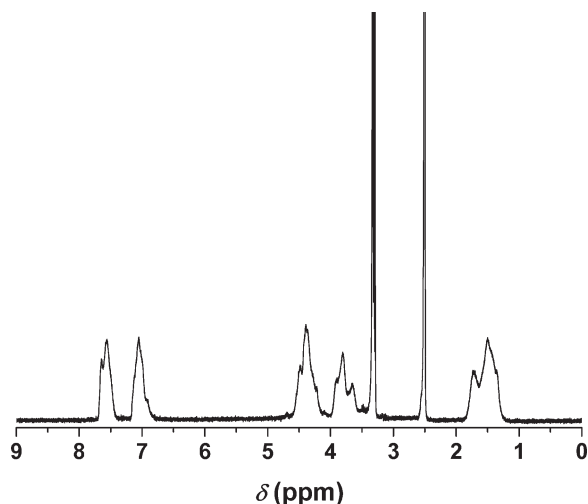
Figure 2. ^{1}H NMR spectrum of MIB-PVAL 13 kDa in DMSO-*d6*.

for 1 month. The nanoparticles were then collected by centrifugation for 20 min with an Eppendorf Centrifuge 5417C rotating at 14,000 rpm and dried at room temperature. The supernatant was analyzed by UV absorbance in order to detect any soluble material that could have leaked out of the particles and the dry solid was analyzed by ^{1}H NMR in DMSO-*d6* solution.

RESULTS AND DISCUSSION

Synthesis of Iodobenzyl Ethers of PVAL

Attachment of both mono- and tri-iodobenzyl grafts to PVAL backbone was performed by the Williamson etherification reaction of the corresponding bromide with the alcohol groups of the PVAL polymer in dry NMP solvent (Figure 1). Ground and dried NaOH, as base, was essential for the etherification reaction, with an addition at last. Purification required several precipitation steps in methanol in order to remove the residual iodobenzyl bromide to completion.

The analyses of the iodinated polymers MIB-PVAL and TIB-PVAL were identical for all derivatives of PVAL of various molar masses. Figures 2 and 3 give illustrative examples of the ^{1}H NMR spectra of MIB-PVAL 13 kDa and TIB-PVAL 13 kDa, respectively. The grafted iodinated aromatic moieties appeared as broad peaks in ^{1}H NMR spectra because the molecular motions of polymers are slow. Conversely, nongrafted reagents and side products appeared as sharp lines. Purification steps were repeated until the sharp peaks had vanished from the ^{1}H NMR spectrum.

The assignment of ^{1}H NMR lines is given in Table I. Grafting aromatic moieties as lateral groups caused a shift of the lines of the polymer backbone, so that the CH and OH lines of PVAL were no longer resolved in the iodinated polymers. Those lines and that of the benzyl CH$_2$ of the iodinated side groups were also poorly resolved.

The degree of substitution, DS = $x/(x + y)$, was calculated by comparison of the area of NMR lines pertaining to the monoiodobenzyl graft (aromatic lines at 7.05 and 7.55 ppm corresponding to two protons each) or triiodobenzyl graft (aromatic lines at 7.60 and 8.04 ppm corresponding to one proton each) and lines

of the PVAL backbone. There are several lines of the PVAL backbone (CH$_2$ between 1.3 and 2.0 ppm; CHOH between 3.4 and 4.5 ppm), so that there are several ways of calculating DS. The CHOH lines of the polymer backbone over a rather wide spectral range extending from 3.5 to 5.0 ppm.[45,46] The CH$_2$ lines of the benzyl groups between 4.1 and 4.7 ppm were not fully resolved because they overlapped the CHOH lines, so that it was difficult to assess the DS from their area. A better accuracy was achieved using the CH$_2$ of the polymer backbone between 1.3 and 2.0 ppm. It was checked that the full area between 3.4 and 4.7 ppm was in agreement with the remainder of the spectra. The substitution degrees of MIB-PVAL 13 kDa and TIB-PVAL 13 kDa were 0.57 and 0.53, respectively. The iodine content was calculated from the substitution degree (Eq. (1)), giving iodine contents in MIB-PVAL 13 kDa and TIB-PVAL 13 kDa of 43 wt % and 68 wt % respectively.

$$\%I = \frac{DS \times M_{(iodine)} \times n}{M_{(nongrafted)} \times (1 - DS) + M_{(grafted)} \times DS} \times 100 \quad (1)$$

wherein $M_{(iodine)}$ is the atomic mass of iodine atom: 127 g mol^{-1}, n is the number of iodine atoms per benzyl group: 1 or 3, $M_{(nongrafted)}$ is the molar mass of nongrafted repeat unit: 44 g mol^{-1}, and $M_{(grafted)}$ is the molar mass of grafted repeat unit: 260 g mol^{-1} for MIB-PVAL and 512 g mol^{-1} for TIB-PVAL.

Elemental analyses of the iodinated derivatives of PVAL 13 kDa confirmed the iodine content calculated from the substitution degree assessed from the ^{1}H NMR spectra, which validated the calculation method of the DS from NMR (Table II). The degrees of substitution of PVAL iodinated derivatives of different molar masses (Table II) were similar, showing that full conversion of the accessible hydroxy groups of PVAL has been achieved in all instances. The DS was probably limited by the steric hindrance of the bulky iodobenzyl groups attached on the polymer backbone. On this basis, the bigger 2,3,5-triiodobenzyl group limited the DS to a lower value than the 4-iodobenzyl group.
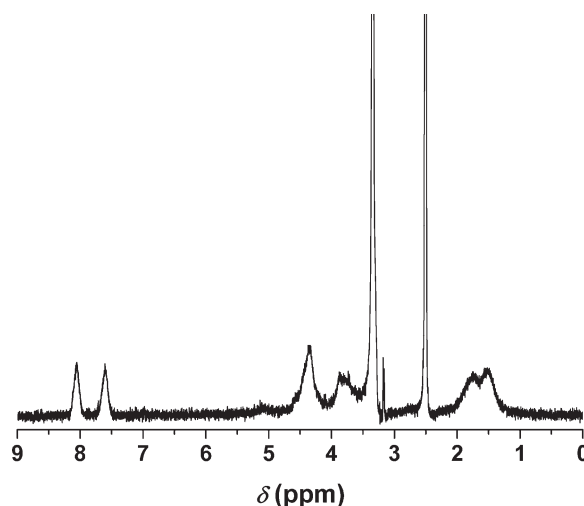

Figure 3. ^{1}H NMR spectrum of TIB-PVAL 13 kDa in DMSO-*d6*.

Table I. Assignment of the ^{1}H NMR Lines of the Iodinated Polymers Valid for PVAL Derivatives of All Molar Masses

Polymer	Chemical shift (ppm)	Area	Assignment
PVAL	1.3–2.0	2	CH$_2$ of polymer backbone
	3.5–4.0	1	CH of polymer backbone
	4.0–4.5	1	OH of polymer backbone
MIB-PVAL	1.3–2.0	2	CH$_2$ of polymer backbone
	3.4–4.5	2-DS	CH and OH of polymer backbone
	4.1–4.7	2DS	CH$_2$ of 4-iodobenzyl group
	7.05	2DS	Ortho protons of aromatic group
	7.55	2DS	Meta protons of aromatic group
TIB-PVAL	1.3–2.0	2	CH$_2$ of polymer backbone
	3.4–4.5	2-DS	CH and OH of polymer backbone
	4.1–4.7	2DS	CH$_2$ of 4-iodobenzyl group
	7.60	DS	H4 proton of aromatic group
	8.04	DS	H6 proton of aromatic group

The IR spectra of the MIB-PVAL and TIB-PVAL showed the expected characteristic bands: the broad band of the O—H vibration centered at 3350 to 3400 cm^{-1}, the stretching vibrations of aliphatic and aromatic C—H between 2950 and 2850 cm^{-1}. The bands below 1600 cm^{-1} were obscured by the numerous aromatic vibrations. In particular, the aralkyl ether bond formed upon the grafting reaction gives two strong IR bands in the regions 1230 to 1270 cm^{-1} and 1060 to 1150 cm^{-1} that could not be distinguished unambiguously. The presence of residual alcohol groups from the partial substitution of PVAL with iodobenzyl groups was detected through several C—OH bands between 1200 and 1400 cm^{-1}. Owing to the high mass of the iodine atom, the aromatic C-I stretching vibration could not be detected because it is shifted out of the mid-infrared window to the far IR (200–400 cm^{-1}). The IR spectra are given in Figure S1 of the Supporting Information together with the list of characteristic bands in Supporting Information Table S1.

Table II. Radiopaque Iodinated PVAL: Degree of Substitution, Iodine Content Determined by ^{1}H NMR and by Elemental Analysis

Grafted polymer	Degree of substitution, DS	Iodine content from ^{1}H NMR (wt %)	Iodine content from elemental analysis (wt %)
MIB-PVAL 13 kDa	0.57	43	43
TIB-PVAL 13 kDa	0.53	68	64
MIB-PVAL 47 kDa	0.61	44	
TIB-PVAL 47 kDa	0.54	69	
MIB-PVAL 61 kDa	0.56	43	
TIB-PVAL 61 kDa	0.46	67	

Synthesis of Iodobenzoyl Esters of PVAL

The corresponding esters of PVAL were synthesized according to a method of the literature[26–28] (Figure 4) in order to compare their stability with that of the ether derivatives. The ^{1}H NMR data were interpreted in the same way as for the iodobenzyl ethers of PVAL. However, the lines in the range 3.5 to 5.0 ppm were still poorly resolved; there was no line of protons belonging to the iodinated grafts in this spectral domain. The DS was calculated in the same way from the areas under the aromatic lines and the CH$_2$ lines of the PVAL backbone between 1.3 and 2.0 ppm. The degree of substitution of the 4-iodobenzoyl ester derivative of PVAL 47 kDa was DS = 0.36 and that of the 2,3,5-triiodobenzoyl ester derivative of PVAL 13 kDa was DS = 0.34. The syntheses of the ester derivatives of PVAL were not optimized towards a maximum DS.

Properties of the Iodinated Polymers

Precipitation Behavior. To prepare the liquid embolics, NMP and DMSO were selected as water-miscible organic solvents for solubilization of the grafted PVAL.[47] Dissolution was performed at room temperature under stirring. Samples were precipitated in water using a syringe with a needle of 0.8 mm internal diameter. The concentrations were chosen in the 10 to 50 wt % range. For TIB-PVAL 13 kDa, only NMP was used because of
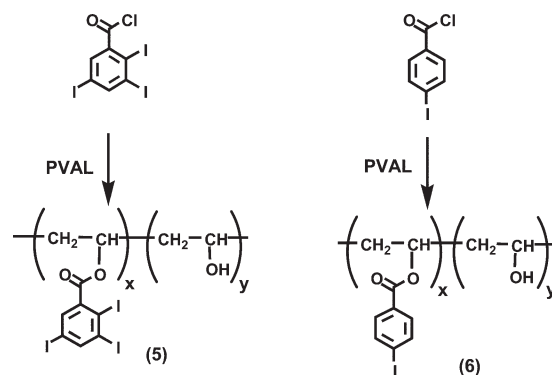

Figure 4. Synthesis pathway of the iodobenzoyl esters of PVAL.

Table III. Precipitation Behavior of Iodinated PVAL in Deionized Water

Grafted polymer	Organic solvent	Concentration (wt %)	Precipitate structure
MIB-PVAL 13 kDa	DMSO	20	Flaky
		30	Hard
		40	Hard
	NMP	20	Flaky
		30	Soft
		40	Soft
MIB-PVAL 47 kDa	DMSO	20	Flaky
		30	Hard
		40	Hard
	NMP	20	Flaky
		30	Soft
		40	Soft
MIB-PVAL 61 kDa	DMSO	20	Flaky
		25	Hard
		30	Hard
		40	Hard
	NMP	20	Flaky
		25	Soft
		30	Soft
		40	Hard
TIB-PVAL 13 kDa	NMP	10	Flaky
		33	Hard

its poor solubility in DMSO. Table III reports data concerning the precipitation behavior. For all iodinated polymers, a high concentration of 50 wt % or above gave solutions that were too viscous for being manipulated with a syringe. Precipitation from solutions of low concentrations between 10 wt % and 20 wt %, yielded pieces of precipitated materials as flakes, instead of the single coherent mass expected for the embolization purpose. All solutions of MIB-PVAL prepared in DMSO at concentrations above 30 wt % gave satisfactory precipitation behavior: a hard and coherent mass of polymeric material formed at the tip of the syringe needle as the DMSO solution was mixed with water. All solutions of TIB-PVAL dissolved in NMP at a concentration above 30 wt % also gave coherent masses of precipitated material. The precipitated masses of MIB-PVAL appeared soft, excepted for the MIB-PVAL 61 kDa precipitated from a 40 wt % NMP solution that was hard. The maximum practical concentration was 40 wt %, as the too high viscosity reached above this concentration did not allow the solution handling with a syringe.

The mechanical properties of the precipitated mass also matters for the polymer plug actually causes an embolization of blood vessels. The mechanical properties of the pure polymers were satisfactory with that respect. Soft semisolid materials were obtained as a result of the precipitation process when part of the solvent remained trapped inside the precipitated mass of polymer. Precipitation into a soft gel typically involves liquid-liquid phase separation into polymer-rich and polymer-poor

phases,[48] the polymer-rich phase being the soft precipitated mass and the polymer-poor phase being the fluid aqueous medium. At low polymer concentration, demixing caused the formation of an aqueous suspension of polymer particles that possibly aggregated into larger particles, precipitation occurred into flakes that are not suitable for embolization. Polymer concentrations above 30 wt % caused the formation of a coherent/cohesive mass of polymeric material. Solutions in NMP led to softer polymer gels compared with precipitation from DMSO solutions because NMP had a higher affinity with the polymer than DMSO. Swelling by NMP may be related to the lower water-affinity and higher viscosity of NMP solutions compared with DMSO. After liquid-liquid phase separation has started, further diffusion of either DMSO or NMP into the aqueous phase may cause the formation of a rigid skin at the surface of the polymer-rich phase.[49] When such a phenomenon is operating, a transitory inhomogeneous material forms with a soft core where the organic solvent is retained, surrounded by a hard shell (skin) that acts as a barrier to diffusion of the organic solvent.

Viscosity of Solutions. The viscosity is a crucial parameter with regards to the application of embolization. Low viscosity in the 30 to 150 mPa s range is desired for embolization of vascular malformations and small capillaries, whereas high viscosity (400 mPa s and above) is more adequate to fill larger structures such as aneurysms. The viscosity of the formulation depends on the concentration of polymer (Figure 5) and type of organic solvent. A concentration range was searched for according to two following mandatory criteria: (i) precipitation allowed formation of a coherent polymer mass; and (ii) the viscosity was low enough for the solution to flow through the 0.8 mm diameter needle of the syringe. A third criterion for the selection of polymer type and organic solvent is the therapeutic application, that is, nature of the site of embolization.

Viscosity of solutions obviously increased with respect to the concentration of polymer. However the viscosity leveled off at high concentrations in few instances. Thus, the viscosity of TIB-PVAL 13 kDa solutions in NMP remained constant (113 mPa s)
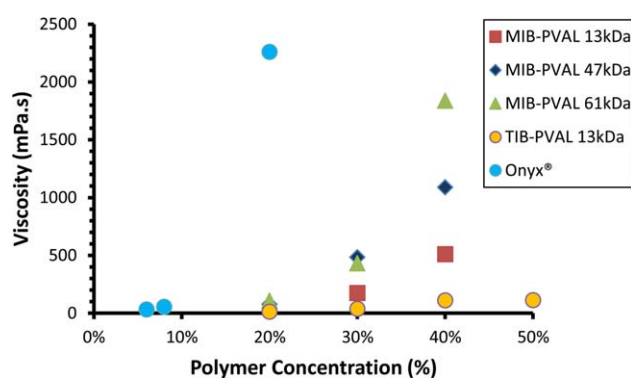

Figure 5. Viscosity of iodinated polymer solutions of various concentrations in NMP. The viscosities of commercial products of the Onyx® series (Onyx®18 containing 6% of poly(ethylene-co-vinyl alcohol) (EVOH), Onyx®34 containing 8% of EVOH and Onyx®500 containing 20% of EVOH) are given for the sake of comparison. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

above a concentration of 40 wt %. Such phenomenon was probably due to the limited solubility of the polymer. The solution at 50 wt % concentration was slightly turbid, indicating that part of the polymer did not dissolve. This was quite difficult to distinguish by visual observation a clear solution where dissolution reached completion and incomplete dissolution where part of the polymer remained as a suspension of translucent particles of small size. Indeed, such insoluble particles often referred to as "microgel" particles are extensively swollen by the solvent, so that their refractive index is close to that of the solvent. Viscosity measurements as a function of polymer concentration provide a sensitive method for assessing the solubility of the polymer: the viscosity remains constant once the solubility has been exceeded. TIB-PVAL 13 kDa solutions in NMP were quite fluid. In comparison, solutions of MIB-PVAL exhibited higher viscosities, even for the low molar mass polymers. As example, the viscosity of the solutions of MIB-PVAL 13 kDa were 318 mPa s and 1450 mPa s for concentrations 40 wt % and 50 wt % respectively, against a maximum viscosity of 113 mPa s for TIB-PVAL 13 kDa. Increasing the molar mass of the PVAL caused a dramatic increase of the viscosity. Indeed, the viscosity of 40 wt % solutions of MIB-PVAL 13 kDa, 47 kDa, and 61 kDa were 512 mPa s, 1089 mPa s, and 1838 mPa s, respectively. However, MIB-PVAL 13 kDa solutions showed lower viscosity at a given concentration when dissolved in DMSO than in NMP. MIB-PVAL 61 kDa solutions prepared in DMSO and NMP had approximately the same viscosity.

Taking together these results, formulations based on either MIB-PVAL or TIB-PVAL might be used to embolize vascular structures. Concentration of 30 wt % or higher ensure polymer cast formation. PVAL molar mass can be chosen to adjust the viscosity of the injectable liquid. As for the solvent carrier, both DMSO and NMP could be used to formulate liquid embolics. NMP was selected for further testing due to its ability to form softer polymer casts; the deformability of the precipitated mass might better allow the formation of a plug in vascular tissues.

Rationale of Solution Properties in Terms of Thermodynamic Solubility Parameters. Solubility behavior and solution viscosities of MIB-PVAL and PIB-PVAL in DMSO and NMP solvents are related to interactions of polymers and solvents. They are described by means of the regular solution theory in terms of the Hildebrand solubility parameters. The solvent quality is said "good" when its solubility parameter is close to that of the polymer. This reveals strong interactions of the solvent molecules and the polymer segments (repetition units), leading to high solvation of the polymer by the solvent. In such a case, the solubility is high and the solution is highly viscous. The high viscosity of the solutions is due to extensive swelling of the macromolecules by the solvent that causes the polymer coils be expanded. The solvent turns worse as the solubility parameters of the polymer and solvent are shifted. In such a case the solution gets less viscous, and the solubility is lost when the solubility parameters shift is too high.

The solubility parameters of NMP and DMSO are 23.10 MPa$^{1/2}$ and 29.70 MPa$^{1/2}$, respectively.[50] The solubility parameters of MIB-PVAL and TIB-PVAL can be either evaluated from experi-

ments based on solubility, viscosity of light scattering, or calculated from group increment tables.[51] There are several classical calculation schemes that make use of tables of group increments. Unfortunately, the increment for the iodide substituent is not available is most tables (by Hoy, van Krevelen, van Krevelen and Hoftyzer, Hoftyzer and van Krevelen).[51] The increment for iodide appears in the table by Small, but the increment for OH is missing. The most comprehensive table in terms of number of available increments is due to Fedors;[52] it includes the increment for the iodide. But it has been noticed that the solubility parameters calculated from the method of Fedors are in slight excess with respect to others methods and experimental determinations. At the end, the classical method of van Krevelen has been retained. This method allows the calculation of the contributions to the solubility parameters due to dispersion (δ_d), polar (δ_p), and hydrogen-bonded (δ_{hb}) interactions according to the Hansen's scheme.[53] The total solubility parameter is given by

$$\delta^2 = \delta_d^2 + \delta_p^2 + \delta_{nb}^2 \qquad (2)$$

The missing increments for the iodo group have been calculated from the group increments of cohesive energies given by Hansen.[54] Those group increments of cohesive energies (noted $\Delta V \delta^2$) for an iodine group bound to an aromatic carbon are $\Delta V \delta_d^2 = 2000$ cal mol^{-1}, $\Delta V \delta_p^2 = 575$ cal mol^{-1}, and $\Delta V \delta_{hb}^2 = 1000$ cal mol^{-1} ($\Delta V = 35.5$ cm^3 mol^{-1}), giving the increments used in van Krevelen's calculation $F_d = 545$ MPa$^{1/2}$, $F_p = 300$ MPa$^{1/2}$, and $E_{coh,hb} = 4000$ J mol^{-1}.

Since the PVAL polymer is not substituted to completion with iodinated grafts, the solubility parameters are calculated for a "pseudo repeat unit" consisting of DS iodinated units and $(1 - DS)$ nongrafted units. The results are presented in Table IV, together with the solvent quality for the different polymer–solvent pairs.

The solubilizing properties of DMSO and NMP solvents for the polymers were calculated from the distance $\overline{\Delta\delta}$ between polymer and solvent in the (δ_d, δ_p, δ_{hb}) space as[51,53]

$$\overline{\Delta\delta} = \left[\left(\delta_{d,pol} - \delta_{d,solv}\right)^2 + 0.25\left(\delta_{p,pol} - \delta_{p,solv}\right)^2 + 0.25\left(\delta_{hb,pol} - \delta_{hb,solv}\right)^2 \right]^{1/2} \qquad (3)$$

where the suffixes "pol" and "solv" stand for polymer and solvent. Good solubility requires $\overline{\Delta\delta} < 5$ MPa$^{1/2}$.[55]

Thus, TIB-PVAL is not soluble in DMSO because DMSO is a poor solvent for TIB-PVAL ($\overline{\Delta\delta} > 7$ MPa$^{1/2}$). MIB-PVAL is soluble in DMSO because the difference of solubility parameters is less; DMSO is a medium solvent for MIB-PVAL ($\overline{\Delta\delta} \sim 6$ MPa$^{1/2}$). Both MIB-PVAL and TIB-PVAL are soluble in NMP that is a solvent of medium quality for both polymers. The details of the specific interactions can be assessed from the contributions to the solubility parameter, δ_d, δ_p, and δ_{hb}, of the polymers and solvents. The main difference between the monoiodinated and the triiodinated derivatives, MIB-PVAL and TIB-PVAL, stands in the dispersive component δ_d which is significantly higher for TIB-PVAL. Such difference probably comes from the low

Table IV. Solubility Parameters δ, and Their Different Contributions δ_d, δ_h, and δ_{hb} (MPa$^{1/2}$) of Polymers and Solvents, and the Distance of the Polymer/Solvent Pairs $\overline{\Delta\delta}$ (MPa$^{1/2}$) in the (δ_d, δ_h, δ_{hb}) Space that Expresses the Quality of the Solvent with Respect to the Polymer

	MIB-PVAL (DS = 0.57)	TIB-PVAL (DS = 0.53)	NMP	DMSO
δ_d	20.85	23.1	18.0	18.4
δ_p	6.3	6.3	12.3	16.4
δ_{hb}	12.5	13.6	7.2	10.2
δ	25.1	27.5	23.0	26.7
$\overline{\Delta\delta}$ and solvent quality	MIB-PVAL (DS = 0.57)		4.9; good	5.7; medium
	TIB-PVAL (DS = 0.53)		6.7; medium	7.1; poor

Data for NMP and DMSO were taken from Ref. 50.

electronegativity of the iodine atom that causes a high polarizability of the C–I bond. It is expected on this basis that TIB-PVAL shows higher δ_d than MIB-PVAL since the microscopic origin of dispersion interactions involves transient polarization of the materials. The dispersive interactions of the two polar solvents DMSO and NMP with TIB-PVAL are less than with MIB-PVAL, and the solubility of TIB-PVAL is problematic. Owing to its high values of δ_p and δ_{hb}, DMSO is a more polar solvent than NMP, so that the iodinated polymers have a low solubility in DMSO because they are mainly nonpolar.

A coherent mass of solid material formed upon precipitation in cases where the solubility parameters of the solvent and polymer were slightly shifted (the solvent quality was "medium"). This is the case of the MIB-PVAL/DMSO and TIB-PVAL/NMP pairs. In the case of the MIB-PVAL/NMP pair, the solvent was retained inside the polymer mass upon precipitation because the solvent is good; the precipitated material was swollen and appeared soft. The solutions of MIB-PVAL in NMP were highly viscous because NMP is a good solvent whereas the viscosity TIB-PVAL in NMP was lower since NMP is a solvent of medium quality.

The solvents have not been selected on the basis of their solubility parameters; their selection relied on their low toxicity that allows their use in an application to a medical device. A high solubility allows the use of highly concentrated solutions. Optimization of the solubility by matching the solubility parameters of the polymer and solvent is not advisable because the viscosity of concentrated solutions is too high and the precipitated material is soft. A moderate viscosity and favorable mechanical properties of the precipitated mass of polymer requires that the solubility parameters are slightly shifted with respect to each other. Their difference expressed in terms of their distance $\overline{\Delta\delta}$ should be lower than 5 MPa$^{1/2}$ for ensuring solubility in the full concentration range, but it should be high enough for the concentrated solution is fluid and the precipitate is a hard enough piece of material. A value of $\overline{\Delta\delta}$ close to 6 MPa$^{1/2}$ appeared a favorable range that met all requirements of the application to embolics (solubility of concentrated solutions, low enough viscosity, precipitation in water as a hard material).

Stability of Iodinated Materials

First, the stability of the iodinated ethers and esters of PVAL was studied with respect to sterilization process. Solutions of

ether and ester MIB-PVAL 47 kDa 40 wt % in NMP were sterilized by autoclaving at 121°C for 15 min; the solutions were cooled at room temperature; the polymers were recovered by precipitation in water, and analyzed by ^{1}H NMR in DMSO-$d6$ solution. The spectra of the two iodinated polymers were identical to those recorded before sterilization. The degree of substitution remained unchanged and no sharp line corresponding to nongrafted material was observed.

Possible released compounds were also tracked in order to confirm this result. The sterilized polymer was recovered by precipitation in basic water, which could solubilize possible released 4-iodobenzoic acid. The recovered solid was analyzed by ^{1}H NMR in DMSO-$d6$ solution and the aqueous phase was analyzed by UV-Vis absorbance. Once again, the NMR spectra were identical to the initial spectra. For both compounds, the UV spectra of the aqueous phases showed that only NMP has been released in water after the precipitation. According to this study, the grafted polymers withstood the sterilization process. The thermal stability of the materials was assessed by thermogravimetric analyses are shown in Figure S2 of the Supporting Information. The TGA scans of the ether compounds showed a large mass loss caused by their thermal decomposition starting at 295°C for MIB-PVAL and 275°C for TIB-PVAL. The thermal decomposition occurring at a single temperature shows that the materials do not leach parts of molecules into their surroundings until they are fully degraded. The TGA data of the ester derivatives showed thermal decomposition in the same range as the ether derivatives, though occurring at slightly lower temperatures. The onset of thermal decomposition of the ester compounds took place at 275°C for the 4-iodobenzoyl PVAL ester and 237°C for the 2,3,5-triiodobenzoyl PVAL. The thermal decomposition of the triiodo derivatives left 10% of their mass as ashes that remained stable up to 900°C. On the contrary, those residual compounds underwent either thermal decomposition or evaporation at higher temperature as revealed by a second mass loss at 410°C; the full mass was lost at the end. The high thermal stability of MIB-PVAL and TIB-PVAL explains their resistance to the sterilization process.

Another stability study was performed with respect to hydrolysis of ester and ether linkages. For this purpose, nanoparticles of iodinated polymer were prepared in PBS buffer, as degradation can be accelerated by increasing the surface area.[56,57] The ester linkage is well-known for being prone to hydrolysis, whereas the

Table V. Concentrations Used for the Fabrication of the Iodinated Polymer Nanoparticles and Their Characteristics

Iodinated polymer used for nanoparticles formulation	Concentration of iodinated polymer (wt %)	Pluronic® F68 concentration in PBS (wt %)	Particles diameter (nm)	Polydispersity index (PdI)
Ether MIB-PVAL 13 kDa	0.5	0.5	267 ± 1	0.17 ± 0.01
Ester MIB-PVAL 13 kDa	0.5	0.5	240 ± 1	0.19 ± 0.01
Ether MIB-PVAL 47 kDa	0.5	0.5	268 ± 3	0.22 ± 0.02
Ester MIB-PVA 47 kDa	0.5	0.5	248 ± 2	0.19 ± 0.02
Ether TIB-PVAL 13 kDa	0.1	0.75	175 ± 3	0.09 ± 0.01
Ester TIB-PVAL 13 kDa	0.1	0.75	170 ± 1	0.10 ± 0.01

ether linkage is resistant. In practice, polymer degradation in PBS buffer would release either iodinated acid for the esters or iodinated alcohol for the ethers. The expected released fragments are 4-monoiodobenzoic acid for the monoiodobenzoyl ester of PVAL or 4-monoiodobenzylic alcohol for MIB-PVAL, and either 2,3,5-triiodobenzoic acid or 2,3,5-triiodobenzylic alcohol for TIB-PVAL. Nanoparticles using ethers and esters MIB-PVAL (13 kDa and 47 kDa) and TIB-PVAL (13 kDa) were prepared by means of the nanoprecipitation technique. THF was used as the solvent of the polymer and satisfactory results were obtained with Pluronic® F68 as emulsifier since aqueous suspensions of submicron particles were obtained. The concentrations of polymer and emulsifier were chosen such that the final particle sizes were very close (200 nm) in all instances, ensuring similar contact areas of the iodinated polymer and buffer medium. Long-term storage for several weeks showed that the suspensions were stable with regards to coagulation. The formulation for the manufacture of the nanoparticles and physical characterization of iodinated PVAL ether or ester nanoparticles are given in Table V.

Particle size was measured by means of dynamic light scattering. Analysis of the autocorrelation function of the scattered intensity using the CONTIN algorithm gave a monomodal particle size distribution in every case. Therefore the mean diameter of the particles was analyzed by the method of cumulants, giving the z-average diameter and the polydispersity index (PdI). The mean diameter of ether and ester MIB-PVAL particles was 200 to 250 nm, and the PdI was around 0.2, indicating a homogeneous size distribution of nanoparticles. The size of ether and ester TIB-PVAL particles was slightly smaller, about 170 nm, and the PdI reached the lower measurable limit of 0.1, which showed that the particle size distribution was narrower than the resolution of the technique.

After separation of the particles and supernatant by centrifugation, UV absorbance analysis of the supernatant and ¹H NMR analysis of the particles showed that the nanoparticles made with the ether derivatives were stable in PBS over one month. Indeed, the UV spectrum of the aqueous phase did not show any absorbance corresponding to the iodobenzyl group. On the contrary, the nanoparticles of the ester derivatives released iodobenzoic acid that was detected by its UV absorbance (Figure 6) as already reported for the similar ester of PVAL poly(vinyl benzoate) by Labruère et al.[58] The amount of released acid calculated for MIB and TIB esters was about 4 mol %.

As expected, the ether linkage revealed more stable to hydrolysis than the ester as no degradation was observed with ether-based iodinated polymers.
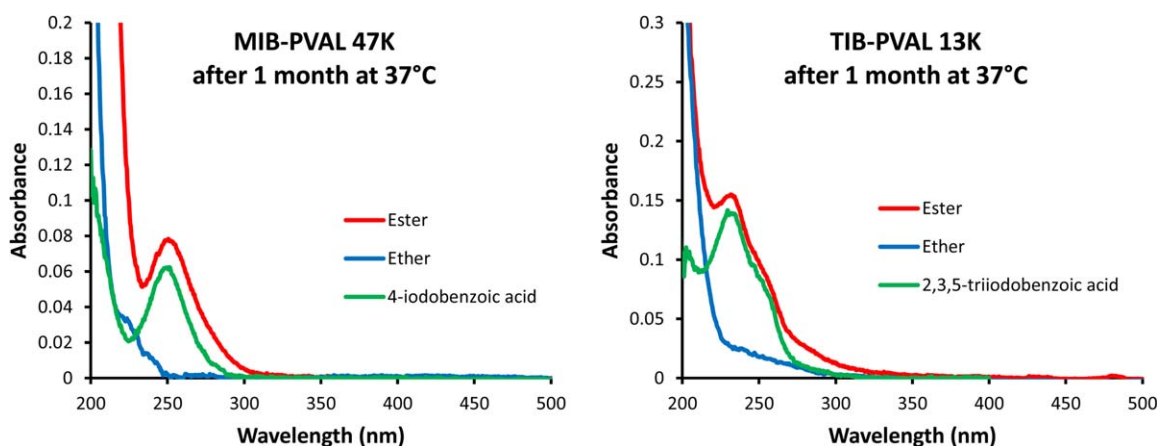

Figure 6. Absorption spectra of the liquid phase of nanoparticles dispersions collected by centrifugation after 1 month storage in PBS pH 7.4 at 37°C. The ester derivatives of PVAL released the corresponding acid as shown by comparison of the spectra of the supernatant and a solution in PBS of either 4-iodobenzoic acid $4.2 \times 10^{-5} M$ or 2,3,5-triiodobenzoic acid $5.0 \times 10^{-5} M$. The ether derivatives of PVAL did not release any molecule that could be detected by UV-Vis absorbance. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
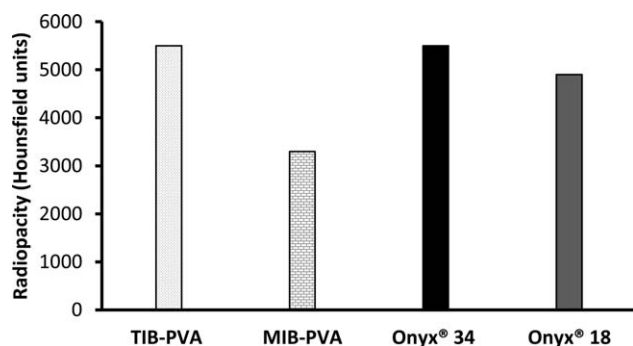
Figure 7. Radiopacity of liquid embolics. Concentrations of TIB-PVAL and MIB-PVAL in NMP were 33 wt %.

Radiopacity and *In Vitro* Assessment of Embolization in an Aneurysm Model

Radiopacity. All formulations displayed a very high opacity to X-rays (Figure 7), three to fivefold higher than that of bones. The radiopacity of the composition containing 33 wt % of TIB-PVAL 13 kDa in NMP was comparable to that of commercial liquid embolizing compositions (Onyx® 34 and Onyx® 18) containing 20% of radiopaque tantalum. The embolizing composition containing 33 wt % of MIB-PVAL 13 kDa in NMP showed lower radiopacity as expected from its lower iodine content. The radiopacity ratio of MIB-PVAL to TIB-PVAL is 61%, which correlates with ratio of the iodine contents of 63% calculated from ^{1}H NMR. Noteworthy, the tantalum particles present in Onyx® settled if left at rest for more than for 1 or 2 min, leading to a highly inhomogeneous distribution of the radiopacity. In contrast, iodinated polymers lead to homogeneous (RDS < 5%) radiopacity throughout the liquid formulation. From these data, it is expected that a 55 wt % solution of MIB-PVAL would have a radiopacity comparable to the Onyx® formulations.

Embolization. As a proof-of-principle, three formulations that allow the formation of a coherent polymer mass were tested for their ability to fill an aneurysm model. Injectable compositions based on MIB-PVAL and TIB-PVAL of the same molar mass (13 kDa) and concentration of 33 wt % in NMP were com-

pared. In addition, the behavior of a higher molar mass MIB-PVAL (47 kDa) was also assessed.

All formulations had the ability to completely fill the 0.5 cm^3 spherical artificial aneurysm with a compact mass of polymer (Figure 8). However, the MIB-PVAL 13 kDa released some polymer particles in the saline flow, indicating an insufficient cohesion of the precipitating polymer mass under shearing by the liquid flow. In a clinical situation, such leaking in the parent blood flow may lead to distal embolization, thus the MIB-PVAL 13 kDa formulation was discarded. In contrast, precipitation of the MIB-PVAL of higher molar mass (47 kDa), as well as the TIB-PVAL 13 kDa, did not lead to any visible leaking of material in the draining flow and the saline fluid did not get turbid. After a period of 3 min, a cohesive mass was formed in the sphere. The commercial compositions of Onyx® behaved in a very similar way, although the final mass showed a more plastic behavior than the TIB-PVAL.

Therefore, both mono-iodinated and tri-iodinated ethers of PVAL with minimal molar mass could be used to design potential liquid embolics for aneurysm filling. Formulation properties can be adjusted through changes of concentration and molar mass of starting PVAL. Higher molar mass polymers generally lead to more cohesive polymer casts, at the cost of reduced syringeability. Conversely, considering their relatively low viscosity at useful concentrations, low-molecular polymers, especially the tri-iodinated ones, may also be used to formulate embolization liquids for small structures such as arteriovenous malformations.

CONCLUSION

Intrinsically radiopaque iodobenzyl ethers of PVAL combining key properties of solubility in water-miscible organic vehicles and insolubility in water hold promises for the embolization of pathological vascular structures under X-ray imaging. Mono-iodinated and tri-iodinated moieties were successfully grafted onto poly(vinyl alcohol), taking benefit of the proven safety record of the backbone polymer. Ethers proved to be highly stable, a guarantee for visualization of the *in situ* formed implants expected to stand for a long duration in the body. The properties of injectable formulations intended for vascular
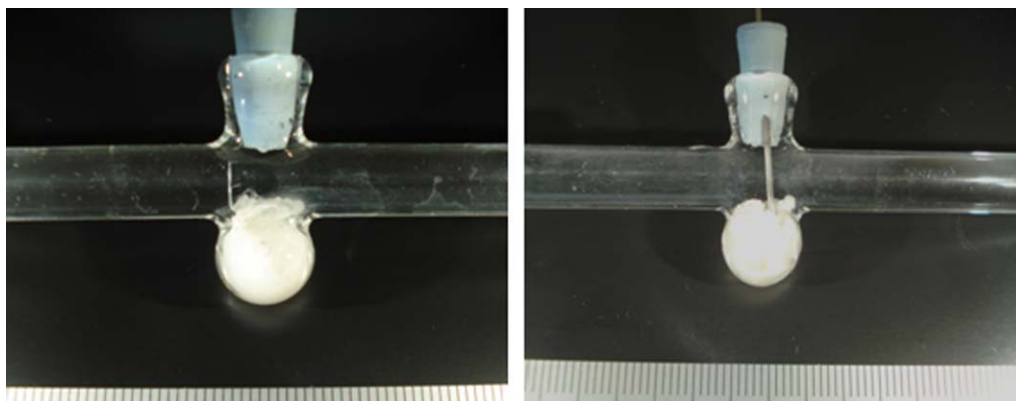

Figure 8. Embolization of the aneurysm model with (left) a 33 wt % solution of MIB-PVAL in NMP; (right) a 33 wt % solution of TIB-PVAL in NMP. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

embolization could be tailored by varying the polymer molar mass, polymer concentration or the nature of the grafted radiopaque group and degree of substitution, in order to target different vascular pathologies under optimized conditions. Besides embolization, nanoparticles, microparticles, or coatings might be obtained to attend medical needs where a permanent, high radiopacity is mandatory to ensure precise imaging and follow-up using modern fluoroscopy or computerized tomography techniques.

REFERENCES

1. Jordan, O.; Doelker, E.; Rüfenacht, D. A. *Cardiovasc. Intervent. Radiol.* **2005**, *28*, 561.

2. Jordan, O.; Rufenacht, D. A.; Iselin, C.; Doelker, E. *Chimia* **2005**, *59*, 353.

3. Mottu, F.; Rüfenacht, D. A.; Doelker, E. *Invest. Radiol.* **1999**, *34*, 323.

4. Purohit, P. J.; Bhat, B. C.; Kulkarni, M. B. *Popular Plastics Packaging* **2005**, *50*(9), 77.

5. Cabasso, I. In Encyclopedia of Polymer Science and Technology; 4th ed.; Mark, H. F., Ed.; Wiley: New York, **2011**.

6. Jayakrishnan, A.; Thanoo, B. C. *J. Appl. Polym. Sci.* **1992**, *44*, 743.

7. Aldenhoff, Y. B. J.; Kruft, M.-A. B.; Pijpers, A. P.; van der Veen, F. H.; Bulstra, S. K.; Kuijer, R.; Koole, L. H. *Biomaterials* **2002**, *23*, 881.

8. Lakshmi, S.; James, N. R.; Nisha, V. S.; Jayakrishnan, A. *J. Appl. Polym. Sci.* **2003**, *88*, 2580.

9. Galperin, A.; Margel, S. *Biomacromolecules* **2006**, *7*, 2650.

10. Galperin, A.; Margel, S. *J. Polym. Sci. Part A: Polym. Chem.* **2006**, *44*, 3859.

11. Zaharia, C.; Zecheru, T.; Moreau, M.-F.; Pascaretti-Grizon, F.; Mabilleau, G.; Marculescu, B.; Filmon, R.; Cincu, C.; Staikos, G.; Chappard, D. *Acta Biomater.* **2008**, *4*, 1762.

12. Constant, M. J.; Keeley, E. M.; Cruise, G. M. *J. Biomed. Mater. Res. Part B: Appl. Biomater.* **2009**, *89B*, 306.

13. Blakely, B.; Lee, B. H.; Riley, C.; McLemore, R.; Pathak, C. P.; Vernon, B. L. *J. Biomed. Mater. Res. Part B: Appl. Biomater.* **2010**, *93B*, 9.

14. Carbone, A. L.; Song, M. J.; Uhrich, K. E. *Biomacromolecules* **2008**, *9*, 1604.

15. Maurer, C. A.; Renzulli, P.; Baer, H. U.; Mettler, D.; Uhlschmid, G.; Neuenschwander, P.; Suter, U. W.; Triller, J.; Zimmermann, A. *J. Hepatol.* **2000**, *32*, 261.

16. Dawlee, S.; Jayabalan, M. *Biomed. Mater.* **2011**, *6*, 055002.

17. Mottu, F.; Rüfenacht, D. A.; Laurent, A.; Doelker, E. *Biomaterials* **2002**, *23*, 121.

18. Mawad, D.; Mouaziz, H.; Penciu, A.; Méhier, H.; Fenet, B.; Fessi, H.; Chevalier, Y. *Biomaterials* **2009**, *30*, 5667.

19. James, N. R.; Philip, J.; Jayakrishnan, A. *Biomaterials* **2006**, *27*, 160.

20. James, N. R.; Jayakrishnan, A. *Biomaterials* **2007**, *28*, 3182.

21. Nisha, V. S.; Joseph, R. *Rubber Chem. Technol.* **2006**, *79*, 870.

22. Nisha, V. S.; Joseph, R. *J. Appl. Polym. Sci.* **2007**, *105*, 429.

23. Dawlee, S.; Jayakrishnan, A.; Jayabalan, M. *J. Mater. Sci.: Mater. Med.* **2009**, *20*, S243.

24. Dawlee, S.; Jayabalan, M. *J. Appl. Polym. Sci.* **2012**, *125*, 2252.

25. Dawlee, S.; Jayabalan, M. *J. Biomater. Appl.* **2013**, *28*, 28.

26. Dudeck, O.; Jordan, O.; Hoffmann, K. T.; Tesmer, K.; Kreuzer-Nagy, T.; Podrabsky, P.; Heise, M.; Meyer, R.; Okuducu, A. F.; Bruhn, H.; Hilborn, J.; Rüfenacht, D. A.; Doelker, E.; Felix, R. *J. Neurosurg.* **2006**, *104*, 290.

27. Dudeck, O.; Okuducu, A. F.; Jordan, O.; Tesmer, K.; Pech, M.; Weigang, E.; Rüfenacht, D. A.; Doelker, E.; Felix, R. *Cardiovasc. Intervent. Radiol.* **2006**, *29*, 1053.

28. Dudeck, O.; Jordan, O.; Hoffmann, K. T.; Okuducu, A. F.; Husmann, I.; Kreuzer-Nagy, T.; Tesmer, K.; Podrabsky, P.; Bruhn, H.; Hilborn, J.; Rüfenacht, D. A.; Doelker, E.; Felix, R. *AJNR Am. J. Neuroradiol.* **2006**, *27*, 1849.

29. Hindenlang, M. D.; Soudakov, A. A.; Imler, G. H.; Laurencin, C. T.; Nair, L. S.; Allcock, H. R. *Polym. Chem.* **2010**, *1*, 1467.

30. Mawad, D.; Lauto, A.; Penciu, A.; Méhier, H.; Fenet, B.; Fessi, H.; Chevalier, Y. *Nanotechnology* **2010**, *21*, 335603.

31. Taki, W.; Yonekawa, Y.; Iwata, H.; Uno, A.; Yamashita, K.; Amemiya, H. *AJNR Am. J. Neuroradiol.* **1990**, *11*, 163.

32. Terada, T.; Nakamura, Y.; Nakai, K.; Tsuura, M.; Nishiguchi, T.; Hayashi, S.; Kido, T.; Taki, W.; Iwata, H.; Komai, N. *J. Neurosurg.* **1991**, *75*, 655.

33. Mandai, S.; Kinugasa, K.; Ohmoto, T. *J. Neurosurg.* **1992**, *77*, 497.

34. Kinugasa, K.; Mandai, S.; Terai, Y.; Kamata, I.; Sugiu, K.; Ohmoto, T.; Nishimoto, A. *J. Neurosurg.* **1992**, *77*, 501.

35. Mottu, F.; Gailloud, P.; Massuelle, D.; Rüfenacht, D. A.; Doelker, E. *Biomaterials* **2000**, *21*, 803.

36. Mottu, F. PhD thesis, University of Geneva: Switzerland, **2000**.

37. Moszner, N.; Saiz, U.; Klester, A. M.; Rheinberger, V. *Angew. Makromol. Chem.* **1995**, *224*, 115.

38. Gailloud, P.; Pray, J. R.; Muster, M.; Piotin, M.; Fasel, J. H. D.; Rüfenacht, D. A. *Surg. Radiol. Anat.* **1997**, *19*, 119.

39. Fessi, H.; Devissaguet, J.-P.; Puisieux, F.; Thies, C. Procédé de dispersion de systèmes colloïdaux dispersibles d'une substance, sous forme de nanoparticules. Patents FR 2608988 (1986); EP 0275796 (1987); US 5118528 (1992).

40. Stainmesse, S.; Orecchioni, A.-M.; Puisieux, F.; Nakache, É.; Fessi, H. *Colloid Polym. Sci.* **1995**, *273*, 505.

41. Yoon, N. M.; Cho, B. T. *Tetrahedron Lett.* **1982**, *23*, 2475.

42. Wullschleger, C. W.; Gertsch, J.; Altmann, K.-H. *Org. Lett.* **2010**, *12*, 1120.

43. Norbash, A.; Singer, R. J. *Am. J. Neuroradiol.* **2001**, *22*, 334.

44. Barnett, B. P.; Hughes, A. H.; Lin, S.; Arepally, A.; Gailloud, P. H. *J. Vasc. Intervent. Radiol.* **2009**, *20*, 507.

45. Wu, T. K.; Ovenall, D. W. *Macromolecules* **1973**, *6*, 582.

46. Moritani, T.; Fujiwara, Y. *Macromolecules* **1977**, *10*, 532.

47. Dudeck, O.; Jordan, O.; Hoffmann, K. T.; Okuducu, A. F.; Tesmer, K.; Kreuzer-Nagy, T.; Rüfenacht, D. A.; Doelker, E.; Felix, R. *AJNR Am. J. Neuroradiol.* **2006**, *27*, 1900.

48. van de Witte, P.; Dijkstra, P. J.; van den Berg, J. W. A.; Feijen, J. *J. Membrane Sci.* **1996**, *117*, 1.

49. Le Renard, P.-E.; Jordan, O.; Faes, A.; Petri-Fink, A.; Hofmann, H.; Rüfenacht, D.; Bosman, F.; Buchegger, F.; Doelker, E. *Biomaterials* **2010**, *31*, 691.

50. Brandrup, J.; Immergut, E. H.; Grulke, E. A. Eds. Polymer Handbook. 4th ed.; **1999**, Chapter 7, p 690, 693,.

51. van Krevelen, D. W.; te Nijenhuis, K. Properties of Polymers: Their Correlation with Chemical Structure; their Numerical Estimation and Prediction from Additive Group Contributions; Elsevier: Amsterdam, **2009**, Chapter 7.

52. Fedors, R. F. *Polym. Eng. Sci.* **1974**, *14*, 147.

53. Hansen, C. M. Hansen Solubility Parameters. A User's Handbook; CRC Press: Boca Raton, FL, **2000**.

54. Hansen, C. M. Hansen Solubility Parameters. A User's Handbook; CRC Press: Boca Raton, FL, **2000**, p. 9, Table 1.1.

55. Hansen, C. M. Hansen Solubility Parameters. A User's Handbook; CRC Press: Boca Raton FL, **2000**, p. 28.

56. Wu, C.; Gan, Z. *Polymer* **1998**, *39*, 4429.

57. Gan, Z.; Fung, J. T.; Jing, X.; Wu, C.; Kuliche, W.-K. *Polymer* **1999**, *40*, 1961.

58. Labruère, R.; Sicard, R.; Cormier, R.; Turos, E.; West, L. *J. Control. Release* **2010**, *148*, 234.