

Synthesis of The Copolymer P(VAc-Co-4VP) For Developing A New SPECT Radioactive Tracer

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Abstract: SPECT is an imaging technique used for diagnostic purposes in Nuclear Medicine. SPECT provides images of superior quality to X-Rays imaging techniques that are normally used during vascular embolization procedures. Vascular embolization is a minimally invasive technique used to reduce the size of a tumor by applying polymeric microspheres. Therefore, radioiodinated polymeric microspheres can be used for SPECT imaging during vascular embolization to make this procedure more efficient and safer. As phenolic compounds allow radioiodination in high yields, these substances can be selected for both copolymerization and radioiodination. In this work, we evaluate the copolymerization between vinyl acetate and 4-vinyl phenol for further radioiodination. Respectively, bulk polymerization tests and conversion study showed that the 4-vinyl phenol must be used at 0.5 % w/w content and the copolymer maximum conversion is reached after 120 min. In addition, this phenolic comonomer affects the polymer mass distribution. Finally, FTIR, RMN, TGA and DSC results showed that the 4-vinyl phenol is present in the polymer backbone. Therefore, these results indicate that the designed copolymer poly (vinyl acetate-co-4-vinyl phenol) (PVAc-co-4VP) was successfully obtained.

Keywords: copolymerization, microspheres, radioactive tracer, radioiodination, SPECT imaging.

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I. Introduction

Copolymers may contain two or more monomers in the polymer chain, resulting in different composition and distribution of the monomers along the polymer backbone. Copolymerization can be used as a strategy to diversify the final polymer properties and end uses. Indeed, most of the commercial polymers are copolymers [1]. Therefore, copolymerization allows the synthesis of a product with specifically desired properties. Bearing this in mind, the insertion of a phenolic comonomer into poly (vinyl acetate) backbone can enhance a radioiodination reaction, for example. According to the literature, the phenol moiety enables the radioiodination of proteins containing phenolic residues of tyrosine or polymer nanoparticles containing phenol, in high yields [2,3]. The 4-vinyl phenol naturally occurs in ciders, wines and berries, and is used as an additive or ingredient in the Food Industry [4-6]. Besides, this substance exhibits both a free unsaturated hydrocarbon chain for the copolymerization and a phenol group for the radioiodination.

Single Photon Emission Computed Tomography (SPECT) technique is widely used in Nuclear Medicine for diagnostic purposes. SPECT uses gamma rays to provide higher resolution images when compared with X-Rays techniques such as Computed Tomography, Angiography and Fluoroscopy [7,8]. Normally, these X-Ray techniques are used during vascular embolization procedures. Vascular embolization is a clinical procedure used to reduce the size of a tumor or to ease its removal during surgery. This procedure consists of injecting fine solid particles dispersed in an aqueous medium, with the aid of a catheter, into blood vessels near the tumor region. In this way, a blockade occurs, and the nutrient supply is interrupted. Over time, the tumor region shrinks and eventually die [9,10].

Polymeric biocompatible and nontoxic particles are used to promote the blood vessels obstruction. For example, poly (vinyl alcohol-co-vinyl acetate) microspheres can be used as an embolic agent. These copolymer particles are obtained through the suspension polymerization and hydrolysis processes where vinyl acetate is the monomer [11,12]. Considering the SPECT high quality images, radioiodinated polymeric microspheres can lead to an innovative embolic agent for SPECT imaging. Consequently, the embolization procedure efficacy can be improved by allowing the track of particles distribution in the veins and tumor, the homogeneity of this distribution and the end of the embolization procedure. This work presents exploratory studies between vinyl acetate and 4-vinyl phenol polymerization to form a copolymer for further radioiodination reaction. Bulk polymerization tests, conversion study and polymer characterization regarding GPC, FTIR, RMN, TGA and DSC analysis were applied in this study.

II. Experimental

2.1. General

The initiator (benzoyl peroxide, BPO, 97 %, containing co-crystallized water 25 %) and the vinyl acetate (VAc) monomer (99.5 %) were supplied by Vetec Química Fina LTDA (Rio de Janeiro, Brazil). The comonomer 4-vinyl phenol (4VP) (10 % in propylene glycol) was supplied by Sigma Aldrich (Rio de Janeiro, Brazil). Tetrahydrofuran (THF, 99.9%) was supplied by Tedia Brasil (Rio de Janeiro, Brazil). Deuterated chloroform (99.80 %) was supplied by Cambridge Isotope Laboratories Inc. (Rio de Janeiro, Brazil). All reagents were used without further purification.

2.2. Comonomer load study

By using bulk polymerization tests, a range of 4VP percentages (Table 1) was studied to define what content of this comonomer should be present in the final copolymer. To perform these tests, VAc and BPO were added in a test tube and mixed until complete homogenization. Then, 4VP was added in this solution resulting in 4 g of the reaction mixture content. After, the test tubes were immersed in an ethylene glycol bath at 80 °C for 2 h. Visual inspection was used to evaluate viscosity variations.

Table 1. Batch of bulk polymerization tests

| | Blank | R01 | R02 | R03 | R04 | R05 | R06 | R07 |
|--------------------|-------|-------|-------|-------|-------|-------|-------|-------|
| BPO (% w/w) | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| VAc (% w/w) | 99.00 | 98.90 | 98.85 | 98.80 | 98.75 | 98.50 | 98.00 | 94.00 |
| 4VP (% w/w) | 0.00 | 0.10 | 0.15 | 0.20 | 0.25 | 0.50 | 1.00 | 5.00 |

2.3. Conversion study

Once the content of 4VP is defined, the reaction time in which the maximum polymerization conversion occurs must also be known. To achieve this parameter, a stock solution (44 ml) containing VAc (98.5 % w/w), BPO (1 % w/w) and 4VP (0.5 % m/m) was prepared. Then, ten test tubes were weighed individually. A 4 mL aliquot of this solution was dispensed into each test tube, which was weighed again. Afterwards, the tubes were inserted into the ethylene glycol bath at 80 °C. At the desired time, each tube was removed from the bath, immersed in an ice bath, to interrupt the polymerization, and dried in a vacuum oven (60 °C and 400 mm Hg) up to constant weight. Finally, mass balance calculations were performed.

2.4. Characterization

Among all samples studied in the conversion, the one corresponding to 150 min of reaction (guarantee of maximum conversion) was characterized by several techniques. Such characterization allowed to determine the specifications of the polymer material and to confirm the occurrence of the desired copolymerization. The blank (PVAc) was also characterized under the same conditions, for comparison purposes.

The molar mass of the obtained polymer was determined by the Viscotek gel permeation chromatography (GPC), model GPC Max, equipped with three Shodex columns (two KF-804L and one KF-805L) and a refractive detector Viscotek VE3580. Calibrations were performed with polystyrene standards ranging from 8×10^2 to 1.8×10^8 Da. The samples were prepared solubilizing 6.00 mg of the material in 3 mL of tetrahydrofuran. This polymer solution was stirred for 24 h and filtered using a PTFE membrane with a 0.20 μm pore diameter. Then, about 200 μL of the solution were injected into the apparatus at 40 °C.

Infrared spectra (FTIR) were obtained in the transmission mode in a Varian 3100 Excalibur Series spectrometer to confirm the polymer chemical structure. The spectra were recorded between 4000-400 cm^{-1} , with 2 cm^{-1} of resolution and 64 scans per analysis using ATR geometry.

Nuclear Magnetic Resonance (NMR) analysis was used to confirm the formation of the copolymer. The sample (~ 250 mg) was solubilized in deuterated chloroform (CDCl_3) (~ 0.6 mL) and stored in an appropriate test tube. ^1H -NMR resonance spectra were obtained using Bruker model AVIII-500 with a frequency of 400.1 MHz and a 5 mm probe.

Analyses of mass losses under heating were performed on TGA Q500 TA Instruments. The analyses were conducted in an inert atmosphere with a constant nitrogen flow at 40 $\text{mL}\cdot\text{min}^{-1}$ and a constant heating rate at 10 $^\circ\text{C}\cdot\text{min}^{-1}$. In addition, the examination of polymer thermal transitions was performed in a DSC equipment Q 1000 TA Instruments. Under nitrogen atmosphere, the thermograms were obtained in the second heating/cooling cycle using a constant heating/cooling rate of 10 $^\circ\text{C}\cdot\text{min}^{-1}$. The first cycle was used for standardization of the samples thermal story.

III. Results And Discussion

3.1. Comonomer load study

According to the viscosity macroscopic evaluation, an increase of viscosity occurred for the experiments from R01 to R05, showing that this range of 4VP content allows copolymerization. In addition, the blank exhibited an increase of viscosity, as expected. Differently, the solution viscosity remained unchanged in the R06 and R07 experiments indicating that no polymerization occurs between VAc and 4VP at these levels. Based on these results, any percentage ranging from 0.10 to 0.50 (% w/w) of 4VP could be used for subsequent studies. However, small amounts of 4VP are more susceptible to errors and may hamper the obtained material characterization. Therefore, 0.50 (% w/w) of 4VP was selected since it is the highest percentage that resulted in copolymerization.

3.2. Conversion study

Fig. 1 shows that the polymerization is slow in the first 90 min of reaction. However, an abrupt and significant increase occurs after this period. With 120 minutes of reaction, the conversion approaches its maximum (90 %) and varies gently around this level. Oliveira and coworkers showed a pure PVAc conversion curve obtained by bulk polymerization, in similar conditions, where the monomer conversion profile follows the usual S-shape trajectory, reaching 94 % after 120 min of reaction [13]. The copolymerization conversion behavior shows that 4VP acts as a polymerization reaction retarder what corroborates with literature data [14,15]. The comonomer 4VP is a phenolic compound and this class of substances acts as a retarder. Moreover, this delay does not imply any impediment to obtaining the desired copolymer.

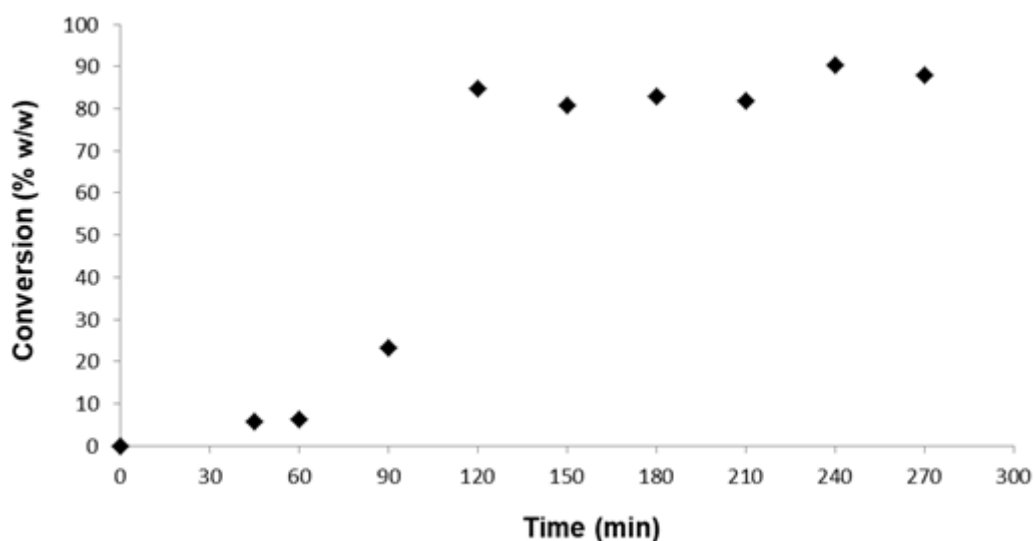
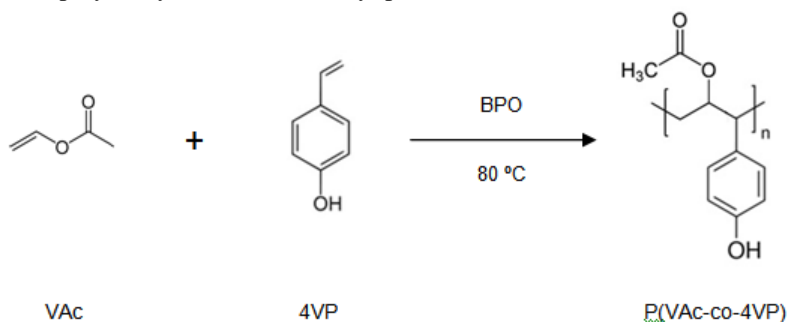


Figure 1. Conversion curve of the bulk polymerization between VAc (98.5 % w/w) and 4VP (0.5 % w/w) performed with BPO (1 % w/w) at 80 °C.

3.3. Polymer characterization

From now on, all the discussions related to the polymer characterization are referred to the likely obtained copolymer, the poly (vinyl acetate-co-4-vinyl phenol) or P(VAc-co-4VP), as shown in Fig. 2.



NOTE: weight ratio VAc / 4VP = 98.50 / 0.50

Figure 2. Polymerization reaction and the most likely chemical structure of the copolymer P(VAc-co-4VP).

3.3.1. Molar mass distribution

GPC analysis results characterized the difference in the copolymer mass distribution related to the presence of 4VP content (Table 2). The number-average molecular weight (Mn), the weight-average molecular weight (Mw) and the polydispersity index (PDI) were the measured parameters in this analysis. Due to the observed increasing of PDI (Table 2), considering that molar mass distribution is affected by the presence of the 4VP comonomer in the polymer backbone seems reasonable. The PVAc obtained in this work has PDI values comparable to reactions that employ a chain transfer agent, while in the presence of 4VP the PDI reaches values compatible with conventional polymerization [13].

Table 2. Mass distribution results and polydispersity index

| | P(VAc-co-4VP) | PVAc |
|----------------|---------------|--------|
| Mn (Da) | 18,902 | 17,989 |
| Mw(Da) | 61,502 | 49,305 |
| PDI* | 3.2 | 1.7 |

*PDI = Mw/Mn

3.3.2. Infrared spectroscopy

Regarding to the infrared area (FTIR), Fig. 3 shows the absorption spectra of the pure PVAc and the copolymer P(VAc-co-4VP) obtained by bulk polymerization. Both samples show similar spectra to the PVAc spectrum reported in the literature [16,17]. These spectra show characteristic absorption bands at 1731 cm^{-1} , corresponding to the axial deformation C=O, and at 1227 cm^{-1} , corresponding to the axial deformation C-O, characterizing the presence of acetate originated from the VAc monomer. The copolymer spectra do not show an intense band at 1500 cm^{-1} , referents to vibrations of aromatic groups. This specific band should be an indicative of the 4VP comonomer presence and its absence can be attributed to the low comonomer content (0.5 % w/w). However, the presence of a wide band at 3500 cm^{-1} refers to -OH stretching vibrations that suggest the 4VP presence. This wide band is a characteristic of the association between hydroxyl (at 4VP) and carbonyl (at VAc) groups by hydrogen bonding, resulting from the polymeric interaction [17,18].

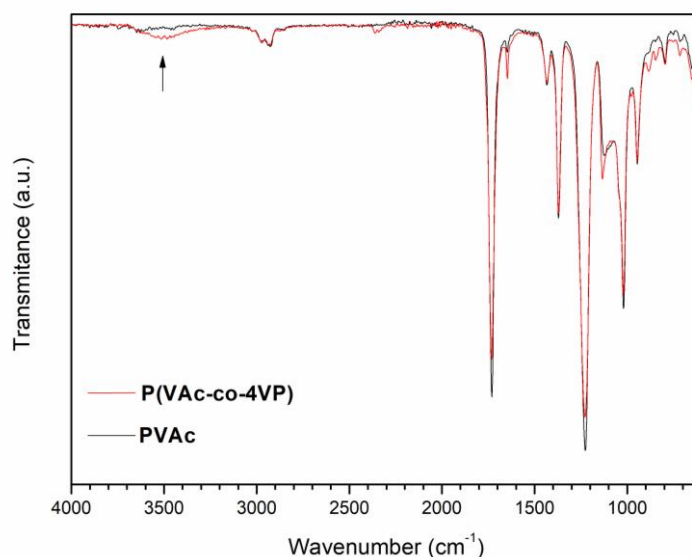


Figure 3. FTIR spectra of PVAc and P(VAc-co-4VP) obtained by bulk polymerization.

3.3.3. ¹H NMR analysis

The ¹H NMR spectrum of PVAc (Fig. 4) is in good agreement with literature reports [19,20]. PVAc characteristic peaks are assigned in the Fig. 4 (a, b and c). The peaks at 1.6-2.2 ppm (a,b) are attributed to CH₂ and terminal CH₃ protons, while the peak of the methine proton (c) is observed at 4.87 ppm. In addition, discrete peaks can be attributed to resulting structures from chain transfer reactions that are commonly observed in the VAc polymerization [19]. The ¹H NMR spectrum of the copolymer contains characteristic peaks of both VAc and PVAc monomers. As the 4VP comonomer is present in low content, its new corresponding peaks exhibits a very low intensity in the spectrum.

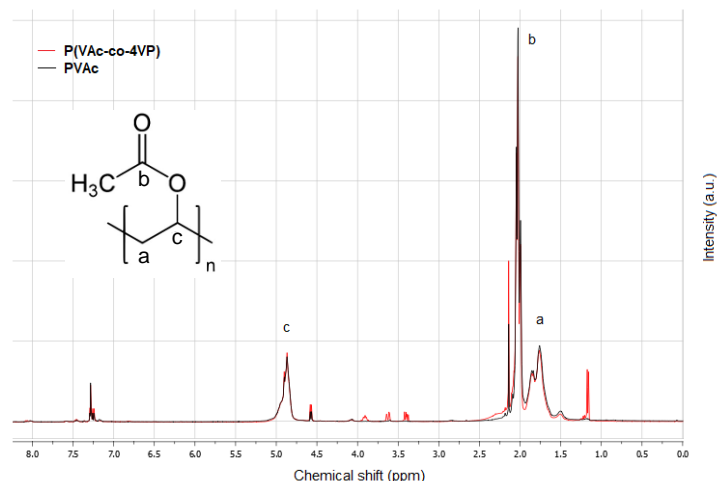


Figure 4. ^1H NMR spectra (400.1 MHz, CDCl_3) of PVAc and P(VAc-co-4VP) obtained by bulk polymerization.

3.3.4. Thermal analysis

TGA and DTG curves of PVAc and the P(VAc-co-4VP) modified polymer are shown in Fig. 5. In essence, both curves present the same behavior, i.e., two thermal degradation temperatures. At 328 °C, the first degradation step is the more intense and is identified as the deacetylation step. After the acetic acid elimination, the slighter degradation observed at a higher temperature (443 °C) is attributed to the decomposition of the remaining PVAc backbone [20, 21]. Comparatively, the degradation of the 4VP (maximum at 399 °C) is confounded with PVAc degradation [22]. Moreover, up to 200 °C, a discrete mass loss indicates a lower thermal stability of the copolymer.

For the pure PVAc and its copolymer P(VAc-co-4VP), DSC thermograms are displayed in Fig. 6. Only one glass transition temperature (T_g) is observed in both thermograms. The T_g value determined for PVAc (29.54 °C) is in good agreement with the value reported in literature [20]. The observation of a single T_g may be evidenced as an indicative of the effective copolymerization. Likewise, a single T_g value may indicate miscibility in polymer blends [18, 23]. Moreover, it can be seen that the copolymer T_g value deviated to the left regarding to the pure polymer one. Correspondingly, this is an expected effect since the insertion of phenol groups increase the mobility of the polymer chain.

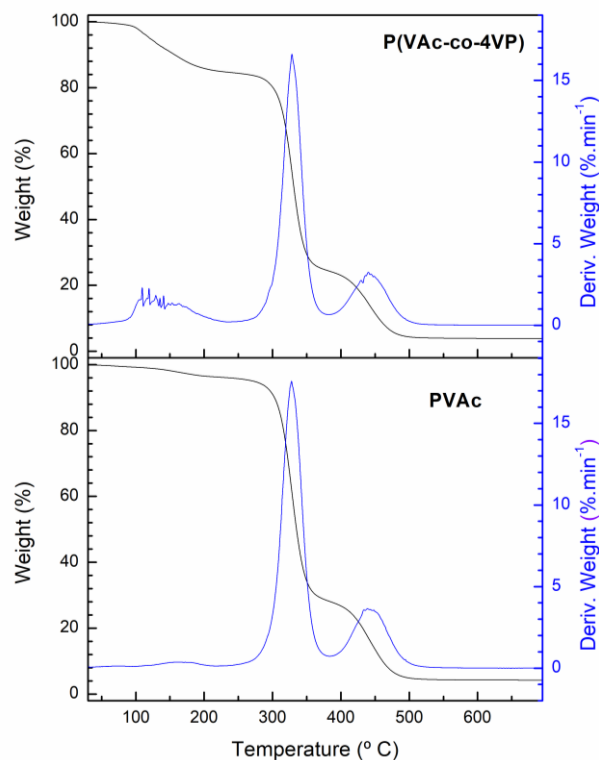


Figure 5. TGA and DTG curves of P(VAc-co-4VP) (0.05 % w/w 4VP) and PVAc.

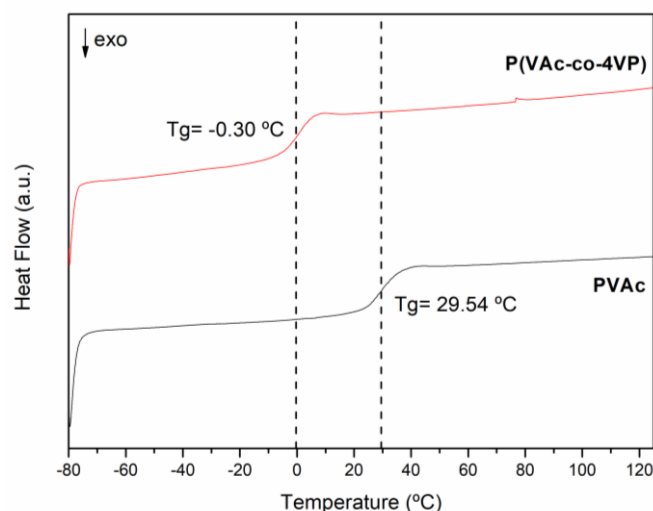


Figure 6. DSC thermograms of P(VAc-co-4VP) (0.05 % w/w 4VP) and PVAc.

IV. Conclusions

A copolymer was designed to provide a new radioactive embolic agent for SPECT imaging purposes. When used in low content (0.5 % w/w), the 4VP showed to be a suitable comonomer to form a polymer with VAc, as confirmed by the characterization results. The obtained copolymer, when compared with the PVAc homopolymer, lead to an increase of polydispersity in the molar mass distribution and a reduction in thermal stability. Overall, to preserve the phenol group in the polymer structure is important to allow further radioiodination. Therefore, the P(VAc-co-4VP) microspheres can be obtained through the polymerization suspension reaction followed by radioiodination in future works.

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