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In-Situ Incorporation of Amoxicillin in PVA/PVAc-*co*-PMMA Particles during Suspension Polymerizations

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Summary: Embolization is a radiological technique that consists in occluding a blood vessel intentionally with an embolic agent (particle). A suspension polymerization process was developed to allow for production of embolic particles with core-shell morphology. This technology was modified to allow for the *in-situ* incorporation of antibiotics (amoxicillin) in PVA/PVAc-co-PMMA core-shell particles. The incorporation of amoxicillin led to modification of some of the final polymer properties, including the particle morphology, the molecular weight distribution and the characteristic transition temperatures of the polymer material. The final polymer properties depended on the antibiotic concentration and on how the drug was added into the polymerization medium.

Keywords: amoxicillin; biological applications of polymers; core-shell polymers; Embolization; suspension polymerization

Introduction

Vascular embolization is a radiological technique that consists in occluding a blood vessel intentionally.[1] During the embolization procedure, an angiographic catheter is introduced into the vascular system and guided to the target, where a fine material is injected as a liquid dispersion, promoting the physical occlusion of the vessel and, consequently, the necrosis of the tissue.[2] The embolization procedure has been used successfully for treatment of leiomyomata, malignant tumors and arteriovenous malformations.[3]

Among the many studied embolic agents, PVA – poly(vinyl alcohol) – particles are the ones used most frequently for its biocompatibility and good embolic performance.[1] Despite that, PVA particles (usually prepared through precipitation from aqueous or alcoholic PVA solutions) present some undesirable characteristics, such as the irregular morphology, tendency to form aggregates (leading to catheter occlusion), fast biodegradability and high cost.[3,4] For this reason, a suspension polymerization process was developed to allow for production of regular spherical PVA/PVAc – poly(vinyl acetate) – embolic particles with core-shell morphology.[4–7]

The proposed suspension polymerization technique comprises two steps. In the first step, spherical PVAc particles are produced through the classical suspension polymerization process. Afterwards, the surface of the PVAc particles is hydrolyzed in a caustic aqueous solution, leading to formation of the PVA shell. The main advantages of this process include the possibility to control the morphology of the PVA/PVAc spherical particles, including the control of the PVA shell characteristics, and the possibility to use a very broad range of operation conditions to manipulate the final particle properties. For instance, comonomers (such as methyl methacrylate, MMA), solvents (such as n-hexane) and charges (such as pharmaceuticals) can be used during the first reaction step to modify the final properties and uses of the produced particles.[4–7]

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Chemoembolization procedures, based on application of embolic agents charged with drugs, have been developed since the 1980's. During chemoembolization, the embolic agent is used simultaneously for the physical occlusion of the blood vessel and for delivery of drugs into the tumoral region.[8–10] Drugs can be incorporated into the polymer particles *in-situ* during the polymerization reaction or through adsorption after preparation of the polymer beads. The *in-situ* incorporation of drugs during the polymerization reaction is usually performed through miniemulsion or precipitation processes.[11–16] Seemingly no studies report the *in-situ* incorporation of drugs during suspension polymerizations to obtain embolic agents. Therefore, it is not known how the *in-situ* incorporation of drugs during PVAc suspension polymerizations can affect the polymerization process and the drug stability and activity. Based on the previous discussion, the main objective of the present work was analyzing how the *in-situ* incorporation of antibiotics (more specifically, of amoxicillin) affects the course of PVAc-co-PMMA suspension polymerizations, intended for production of embolic agents, and the final characteristics of the obtained polymer material.

Experimental Part

Suspension Polymerization

The detailed description of the experimental procedures is described elsewhere.[4–7] First, PVAc-co-PMMA particles were obtained through suspension polymerizations. Reactions were carried out in a 1-L jacketed glass reactor at 90 °C. Initially, the reactor was charged with distilled water, containing a specified amount of suspending agent (PVA). After reaching the desired reaction temperature, a solution of comonomers (vinyl acetate and methyl methacrylate, 70:30) and initiator (BPO) was added. The system was kept under isothermal conditions with a constant agitation of 600 rpm. Reactions were halted after 4 hours. The final PVAc-co-PMMA particles were filtered, thoroughly washed with distilled water and dried under vacuum at 25 °C. Different amounts of amoxicillin were added into the polymerization medium, ranging from 0 to 1.0 wt% in respect to the total mass of the comonomer mixture. Drug addition was performed through the aqueous phase (as a solution, also containing PVA) or through the organic phase (as a suspension/solution in the comonomer mixture) before the beginning of the reaction.

Saponification

In order to produce the PVA shell, obtained PVAc-co-PMMA particles charged with amoxicillin were hydrolyzed in a caustic solution containing NaOH 40 wt% at 30 °C for 2 hours. After the hydrolysis, particles were filtered, thoroughly washed with distilled water and dried under vacuum at 25 °C.

Characterization

Particle morphology was characterized through electron microscopy. The molecular properties of the final polymer samples were characterized through differential scanning calorimetry (DSC), gel permeation chromatography (GPC) and ^{13}C-NMR. The release profile of the drug in aqueous medium was characterized through UV-VIS spectroscopy. The standard characterization procedures used here are described in detail elsewhere.[4–7]

Results

Micrographs of final samples showed that obtained particles presented the usual spherical shape, although the surface characteristics changed when the experimental conditions were changed (Figure 1). It is interesting to observe that the final polymer particles presented a porous surface layer when amoxicillin was incorporated through the organic phase. This indicates that the drug can present surface activity, which can affect the course of the suspension polymerization process and modify the final

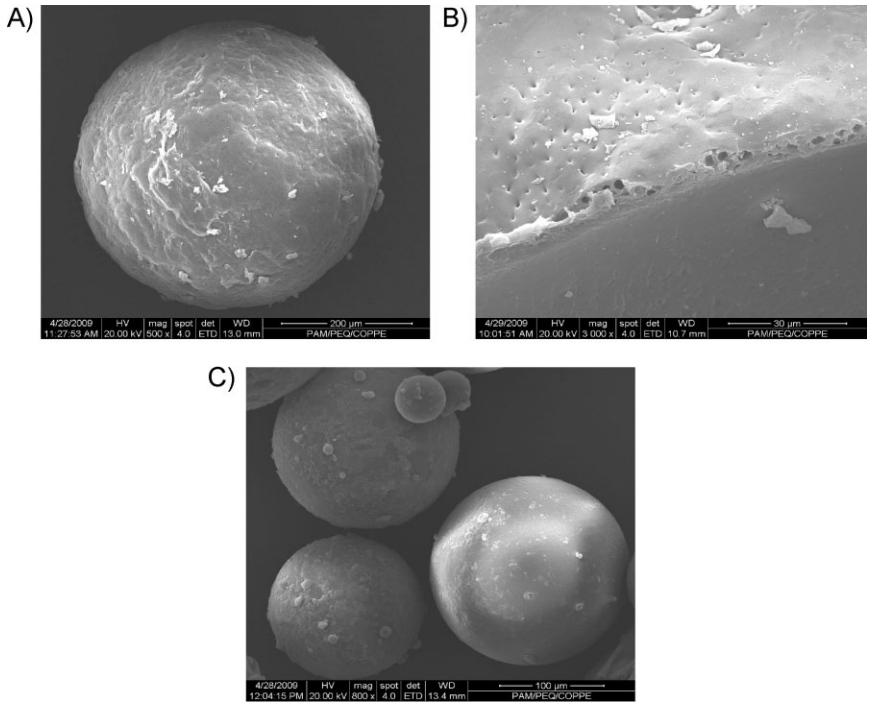

Figure 1.
(A) PVA/PVAc-co-PMMA particles prepared with 1 wt% of amoxicillin, as added through the organic phase. (B) Surface characteristics of PVA/PVAc-co-PMMA particles prepared with 1 wt% of amoxicillin, as added through the organic phase. (C) PVA/PVAc-co-PMMA particles prepared with 1 wt% of amoxicillin, as added through the aqueous phase.

morphological features of the obtained beads. The porous layer was not observed when amoxicillin was incorporated through the aqueous phase, possibly indicating that amoxicillin was incorporated with lower efficiency in this case.

One must consider that the solubility of amoxicillin in the aqueous phase ranges from 2.9 to 15.4 mg/mL (0.3 to 1.5 wt%) when temperature varies in the range between 25 and 70 °C, while the solubility of amoxicillin in VAc ranges from 0.15 to 0.22 mg/mL (0.02 to 0.03 wt%) when temperature varies in the range between 25 and 50 °C. Consequently, when amoxicillin was added through the organic phase, the drug was added into the reactor as a fine powder, suspended in the small monomer droplets. This can explain the accumulation of fine amoxicillin particles in the vicinities of the particle surface and the formation of the porous superficial layer during particle washing. On the other hand, as amoxicillin was completed dissolved in water when it was added into the reactor through the aqueous phase, formation of fine amoxicillin particles should not be expected. It is clear, though, that the surface activity of amoxicillin in the analyzed aqueous-monomer suspensions must be investigated in detail.

Figure 2 shows the glass transition temperatures (T_g) and the average molecular weights (M_w) of polymer samples as functions of the amoxicillin content. As shown in Figure 2, the *in-situ* incorporation of the drug caused significant modifications of both T_g and M_w values, regardless of the technique used to incorporate the drug into the final beads. It is also important to observe that the observed effects are nonlinear and indicate that the drug possibly

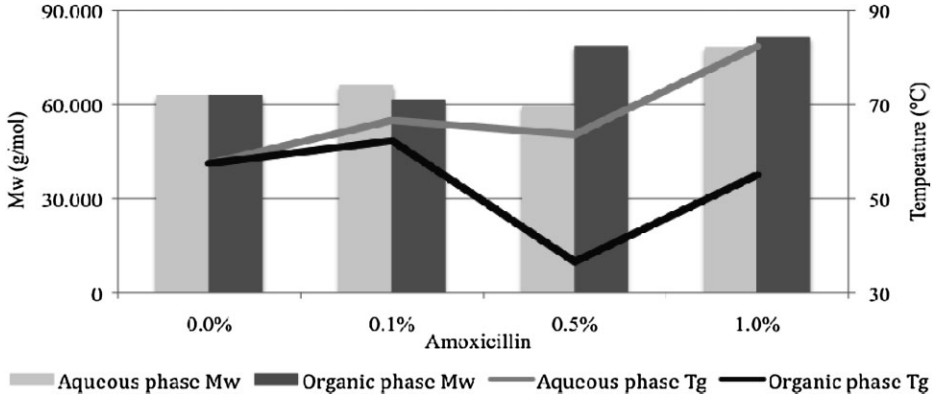

Figure 2.
T_g and M_w values of PVAc-co-PMMA particles as functions of the amoxicillin content and the incorporation method (organic phase and the aqueous phase).

affects the mechanism of polymerization. This assumption can be confirmed in Figure 3, as monomer conversion tended to decrease when the amoxicillin content of the aqueous phase was increased. This shows unequivocally that amoxicillin can be partially absorbed by the suspended polymer particles, affecting the course of the polymerization.

Figure 2 shows that the average molecular weights of the polymer material tended to increase when the amoxicillin content increased, while the T_g values presented a point of minimum. Based on the observed effects, it is believed that amoxicillin can be incorporated into the final polymer chains in small quantities and promote cross-linking of polymer chains. As shown in Figure 4, amoxicillin presents many functional groups that can interact with the growing polymer chains and give support to this assumption.

^{13}C-NMR analyses were performed in order to determine the bead composition and detect the possible incorporation of amoxicillin into the polymer chains. Obtained spectra were very similar to the one presented in Figure 5 in all cases.

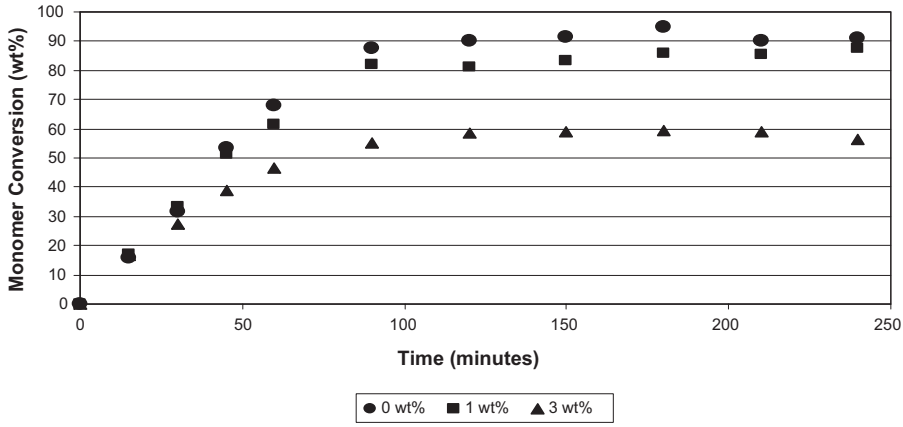

Figure 3.
Evolution of monomer conversion during VAc homopolymerizations when varying amounts of amoxicillin were added through the aqueous phase.

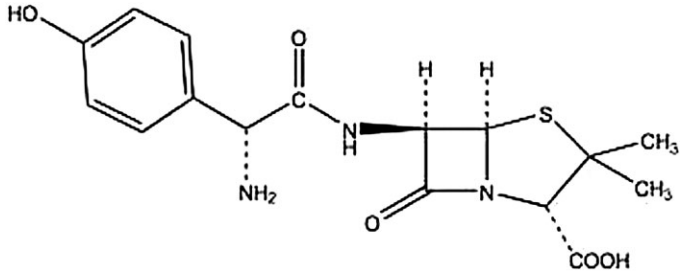

Figure 4.
Molecular structure of amoxicillin.

Figure 5 confirms the existence of PVAc (21.1, 66.9, and 170.3 ppm), PMMA (16.5, 18.7, 44.5, 51.8, 177.8 ppm) and PVA (44.9, 67.9 ppm) blocks, as reported previously.[17] This confirms the formation of the copolymer and of the PVA shell (as discussed in previous works[4–7]). However, despite the very significant modification of the molecular characteristics of the final polymer samples, Figure 5 does not confirm the incorporation of amoxicillin molecules into the final polymer chains. This is probably due to the fact that very low amounts of amoxicillin are incorporated into the final polymer chains, below the detection limits of the NMR characterization technique.

Figure 6 presents the total amounts of amoxicillin detected after drug release experiments and schematic representations that describe how amoxicillin is distributed inside the polymer beads in the distinct analyzed cases. The release tests were performed in aqueous media containing 1 wt% of lauril sodium sulfate at 25 °C, before and after saponification. As shown in Figure 6, significant amounts of amoxicillin were extracted from polymer beads in both cases, showing that part of the drug was not chemically bound to the polymer chains. When the drug was added through the organic phase, it can be assumed that the drug was dispersed in the whole particle (although accumulation of amoxicillin in the particle surface was likely to occur, as discussed previously). This probably explains why the amount of drug released

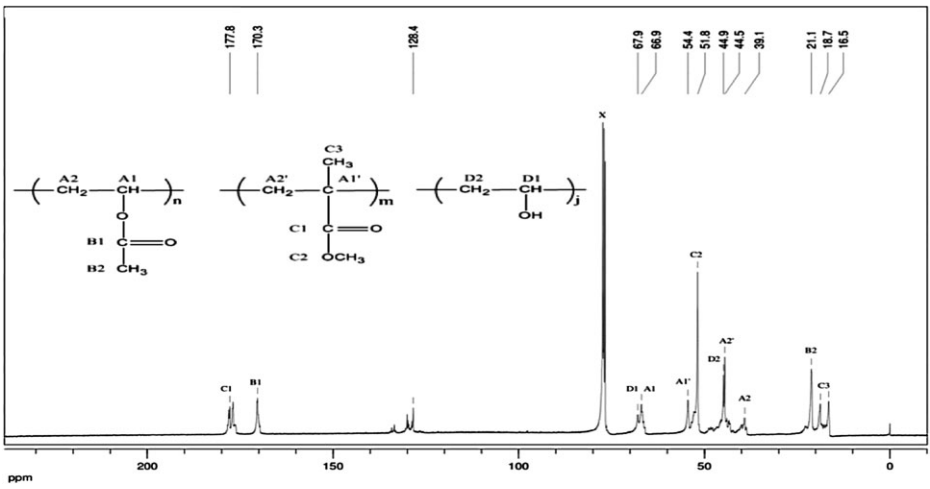

Figure 5.
^{13}C-NMR spectrum of PVA/PVAc-co-PMMA particles containing 1.0 wt% of amoxicillin, as added through the organic phase.

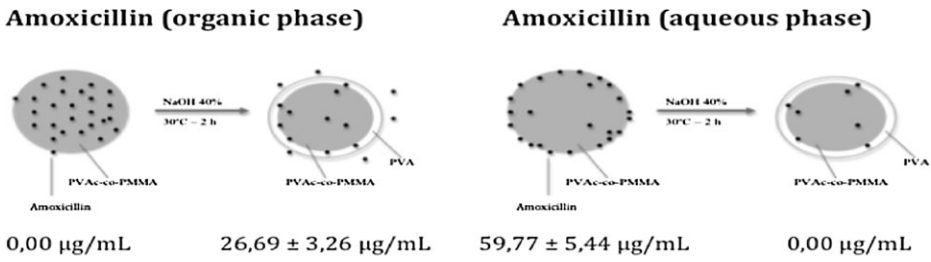

Figure 6.
Schematic representation of the amoxicillin distribution in the final polymer beads before (PVAc-co-PMMA) and after (PVA/PVAc-co-PMMA) saponification.

before saponification was very low, as one might expect the development of significant resistance to drug diffusion in the polymer matrix. However, when saponification was performed, large amounts of amoxicillin were extracted from particles during drug release experiments. This can be explained in terms of the increased porosity of the polymer beads after the partial hydrolysis of the PVAc-co-PMMA chains. When the drug was added in the polymerization medium through the aqueous phase, one could observe the opposite behavior, as higher amounts of amoxicillin could be extracted from polymer particles during release experiments before (but not after) saponification. This probably indicates that amoxicillin was placed primarily on the particle surface, being hydrolyzed or removed from the beads during the saponification step.

Conclusion

Suspension copolymerizations of vinyl acetate and methyl methacrylate were performed in presence of amoxicillin to allow for production of PVA/PVAc-co-PMMA copolymer beads used as chemoembolic agents. It was observed that the *in-situ* incorporation of the drug is indeed possible, although it can promote a series of changes in the final characteristics of the obtained polymers particles, including changes of the final particle morphology (as observed through microscopic analyses) and of the final molecular properties of the polymer material (as observed through characterization of glass transition temperatures and molecular weight distributions). The obtained results depended on how the drug was added into the reaction medium (through the aqueous phase or the organic phase) and on the amoxicillin contents, also indicating that amoxicillin presents surface activity (as it changed the morphological features of the polymer beads even when added in small amounts) and probably modifies the kinetic mechanism of polymerization (as it changed the monomer conversion, the average molecular weights and glass transition temperatures of final polymer samples). Additionally, drug release experiments indicated that part of the drug was not chemically bound to produced polymer chains, as confirmed through ^{13}C-NMR analyses. Drug release experiments also indicated that the release profiles depended on the saponification step: when amoxicillin was added through the organic phase, the saponification step led to significant increase of the amounts of released drug because of the increased porosity of the particle shell; when amoxicillin was added through the aqueous phase, the saponification step led to significant decrease of the amounts of released drug, because of drug removal or hydrolysis during particle saponification.

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