In Situ Incorporation of Doxorubicin in Copolymer Particles During Suspension Polymerization

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Summary: This work reports the kinetic behavior and the molecular properties of obtained copolymers during vinyl acetate and methyl methacrylate suspension copolymerizations in presence of doxorubicin. The obtained spherical particles are intended for use as chemoembolization agents. It is shown that the presence of doxorubicin in the reaction medium promotes significant changes in the copolymerization kinetics and final molecular weight averages of the polymer product.

Keywords: chemoembolization; core-shell particles; doxorubicin; suspension polymerization

Introduction

The term *drug delivery system* was created to describe systems that release drugs continuously, providing the desired pharmacological activity with minimal side effects. The main characteristics required for a controlled delivery system are the ability to absorb the drug and the capacity to release it in a controlled manner, at specific *sites* (target) and in quantities that are sufficient to perform the treatment.[1] Regarding the controlled release of anticancer drugs, some additional properties are also desired, including: (1) capacity to reach the tumor (sick tissue) with great specificity, (2) ability to promote the drug absorption by the sick tissue, (3) capacity to control and maintain the drug release during treatment, (4) increase of the tumor exposure time to chemotherapy, and (5) reduction of the systemic drug levels.[2]

Many studies have been performed to develop drug delivery systems.[3–10] Particularly, polymer materials have received special attention in this field because of their many competitive advantages.[11,12] One of the main advantages is the fact that synthetic polymers can be prepared through several different strategies. Besides, it is possible to control the final *quality* of most polymer materials, so that synthetic polymers can be *designed* to present some desired characteristics, such as biocompatibility and/or biodegradability.

Many methods are used to load drugs (or biological active substances) into polymer matrices, including co-precipitation, in-situ incorporation and adsorption. In the first case, the doped polymer beads are prepared through precipitation from a solution that contains both the drug and the polymer resin. In the second case, the drug is solubilized in the reaction medium before the synthesis of the final polymer, which is formed in the presence of the drug. In the third case, the drug is added to a medium that contains the suspended polymer material after production of the polymer matrix. When the in-situ incorporation technique is performed, entrapment of the drug within the polymeric matrix is expected to occur. On the other hand, when the adsorption technique is performed, the drug is expected to be predominantly on the surface of the carrier device.[5] The in-situ incorporation process can be extremely interesting because it allows for reduction of the production costs by reducing the number of required process steps.

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However, it must be assured that it will be possible to release the drug from the polymer matrix and also that the incorporated drug will preserve its activity.

Most studies related to the in situ incorporation technique are based on emulsion and miniemulsion polymerization processes,[6,13–17] although the in situ incorporation of drugs through suspension polymerizations has also been reported.[7] Despite that, the technique used most often for loading of polymers with *drugs*, including chemotherapic agents, is the adsorption method. Other techniques include solvent solubilization,[18] interfacial deposition,[19] spray-drying [20] and solvent evaporation.[10] A possible alternative is to modify the monomer, so that the final polymer material exhibits pharmacological activity,[21] or covalently-link the desired drug to the polymer matrix.[22]

Vascular embolization is a medical procedure that consists in occluding a blood vessel intentionally by injecting a fine material (embolic agent) intravenously.[23–25] Embolization techniques have been used to treat several different clinical problems for years,[25] including the successful treatment of malignant tumors and arteriovenous malformation.[26] Transarterial chemoembolization (TACE) [27,28] is a medical technique that combines the local application of a chemotherapeutic drug with an embolic agent, leading to local chemical and physical actions in the treated tissue.[29] The idea of combining a chemotherapeutic drug with an embolic agent also brings other important advantages, such as the local drug release – due the ischemic action of the embolic agent – which contributes to reduction of the side effects associated with the chemotherapeutic drugs.[27,30]

Materials used in TACE procedures have to be seen simultaneously as embolic agents and drug delivery systems. As embolic agents, they must present many properties, including the efficient vascular occlusion, biocompatibility, spherical morphology and well-defined size range.[26,31,32] As drug delivery systems, they must be biocompatible, mechanically resistant, capable of achieving high drug loading, simple to administer and easy to fabricate and sterilize.[2] In order to achieve all these characteristics, polymer particles are frequently used both as embolic agents and drug delivery systems, as polymer particles can be produced with a broad range of natural and synthetic materials, with different sizes, shapes, physical states and surface characteristics.[11,12] This explains why most TACE products are based on polymer materials.[33]

Poly(vinyl alcohol) (PVA) and poly(-methyl methacrylate) (PMMA) are two important commercial polymers that are used in a wide range of applications, including medical applications, due to its biocompatibility.[34,35] PMMA can be easily synthesized through direct polymerization of its monomer, methyl methacrylate, in a broad range of systems and operation conditions.[36] However, PVA cannot be synthezied through direct polymerization, as pure vinyl alcohol monomer cannot be obtained. Thus, the commonest route used to produce PVA comprises two steps: first, a vinyl ester based polymer, such as poly(vinyl acetate), is produced; then, the resin is modified (usually hydrolyzed) to produce PVA.[37,38]

In the past years, Pinto and coworkers developed a sequential two-stage process to allow for production of spherical PVAc/PVA particles with core-shell morphology to be used as embolization agent.[39,40] Based on this process, additional studies were carried out to modify the final properties of the particles. In one of these studies,[41] the final density of the particles was manipulated, using solvent and an additional expansion step. Another study[42] showed that it is possible to modify the glass transition temperature (T_g) of the final copolymer from 40 °C to 60 °C, increasing the mechanical stability of the particles, by using 30 wt.% of MMA as comonomer in the VAc suspension polymerization step.

In order to improve the therapeutic effect of the PVAc/PVA embolic parti-

cles,[39,40] drugs can be incorporated into the polymer beads. For this reason, small amounts of amoxicillin (a multifunctional drug used as prophylactic agent during embolization procedures) (0–1 wt.%) were added in situ to VAc suspension polymerization media. It was observed that the presence of the drug caused the production of irregular particles with low T_g values (<40 °C) and high tendency to form aggregates.[43] However, in presence of 30 wt% of MMA it was possible to obtain spherical particles loaded with the drug.[42,44,45] Despite that, it was shown that the in situ addition of amoxicillin during the suspension VAc/MMA copolymerization affected not only the final properties of the polymer particles (particle morphology, molecular weight, copolymer composition and thermal behavior) but also the polymerization kinetics, including additional chain transfer and inhibition steps.

Based on the previous paragraphs, in the present study the kinetic behavior, morphological and molecular properties of copolymer resins obtained during vinyl acetate and methyl methacrylate suspension copolymerization are studied in presence of doxorubicin. The main objective is to evaluate the production of chemoembolic agents through in situ incorporation of doxorubicin during the polymerization.

Experimental Part

Materials

Benzoyl peroxide (BPO, 97%), anhydrous calcium chloride ($CaCl_2$ 96%), hydroquinone (99%), poly(vinyl alcohol) (PVA, $Mw = 78 \times 10^3\ g \cdot mol^{-1}$ and degree of hydrolysis of 85%), sodium hydroxide (NaOH, 99%), and vinyl acetate (VAc, 99%) were purchased from Vetec Química Fina (Rio de Janeiro, Brazil). HPLC grade Tetrahydrofuran (THF, 99.9%) was purchase from Tedia Brazil (Rio de Janeiro, Brazil). Doxorubicin hydrochloride was supplied by Eurofarma (São Paulo, Brazil). Deuterated dimethyl sulfoxide (DMSO-d_6, 99.9%) was purchased from Cambridge Isotope Laboratories (Tedia Brazil, Rio de Janeiro, Brazil). Methyl methacrylate (MMA, 99%) was kindly supplied by Dentsply Brasil (Petrópolis, Brazil). The water used in all experiments was purified by a sequential three-step purification process (distillation, demineralization and micro-filtration). All chemicals were used without further purification except when indicated.

Monomer Purification

Vinyl acetate (VAc) and methyl methacrylate (MMA) monomers were separately purified prior to use. First, the monomer was washed with an aqueous solution of NaOH 3 wt. % (1:1 in volume). The washing procedure was carried out two times. Afterwards, the monomer was neutralized by washing with distilled water (1:1 in volume) until reaching pH of 7. The monomer was transferred to an amber flask containing a small amount $CaCl_2$ and stored at −15 °C for 24 hours. Residue water content was discarded and the precipitated $CaCl_2$ was removed by filtration. Finally, the monomer was distilled under nitrogen atmosphere. The purified monomer was stored at −15 °C until final use.

Suspension Polymerization

Poly(vinly acetate-co-methyl methacrylate) random copolymers (PVAc-co-PMMA) were synthesized in absence and in the presence of doxorubicin, following the basic methodology and recipe (Table 1) described previously,[40,42–45] with some minor experimental adaptations.

Suspension copolymerization reactions were carried out in a 1L jacketed glass reactor, under atmospheric pressure. Initially, the reactor was loaded with purified water, containing the specified amount of suspending agent (PVA). When the desired temperature was reached (70 ± 5 °C), a solution containing the desired amounts of initiator (BPO) and purified comonomers (VAc: MMA) was added into the reactor. The system was kept under isothermal condition with a constant agitation

Table 1.
Basic recipe used to carry out VAc/MMA suspension copolymerization reactions in absence and in presence of doxorubicin.

Organic phase	Aqueous phase	Amount (g)	Note
VAc		140	1.6 mol
MMA		60	0.6 mol
BPO		4	1.6×10^{-2} mol
	Water	420	–
	PVA	0.2	2.6×10^{-6} mol
	Doxorubicin	0 or 0.05	8.6×10^{-5} mol

of 700 rpm. During the reaction, the reactor was closed with its top lid, which was equipped with a reflux condenser. After 240 minutes of reaction, the reactor temperature was reduced to $30 \pm 5\,^{\circ}C$. The resulting precipitated polymer was filtered and washed with distilled water. The final copolymer particles were dried under vacuum overnight at 25 °C. Doxorubicin was added into the polymerization medium, before the beginning of the polymerization reaction. The drug was solubilized in 10 mL of the aqueous phase solution (*water* with PVA; see Table 1), and charged into the reactor. Drug addition was performed only through the aqueous phase of the suspension system.

During polymerization, samples were taken at specified times for characterization. In a disposable aluminum vessel, approximately 4 g of sample were taken from the reactor and mixed with few drops of a hydroquinone aqueous solution (1 wt.%). The weight of each sample was recorded just after the sampling process. After reaching room temperature, all samples were dried at $65 \pm 5\,^{\circ}C$ until constant weight ($\pm 5 \times 10^{-4}$ g).

Saponification

In order to produce a PVA shell at the surface of the particles, obtained PVAc-co-PMMA copolymers were hydrolyzed in a caustic solution containing aqueous NaOH 40 wt.% at 30 °C for 120 minutes. After the hydrolysis, particles were filtered, thoroughly washed with distilled water and dried under vacuum at 25 °C. Additional details are provided elsewhere.[39–41]

Characterization

Scanning electron microscopy (SEM) was used to determine the particle morphology using a Quanta 200 microscopy (FEI Company, USA). In order to perform SEM analysis, polymer samples were covered with a thin layer of gold (±300 nm) on a JFC-1500 ion sputtering device (JEOL, Japan) prior to analysis. Particle morphology was also observed using an optical microscopy (Nikon SMZ 800, Japan).

^{1}H-NMR analyses were performed in a Bruker DPX 300, operating at 300 MHz, using deuterated dimethyl sulfoxide (DMSO-d_6) as solvent. Analyses were performed at room temperature.

Particle size distributions were recorded in a Mastersizer 2000 (Malvern, UK), using a small amount of dry polymer. Before analysis, polymer samples were submitted to gentle manual milling in order to disintegrate polymer aggregates. The operation did not affect the integrity of the beads, as observed through optical microscopy.

Monomer conversion was measured gravimetrically as described in the previous section.

Gel Permeation Chromatography (GPC) analyses were performed in THF at 40 °C and flow rate of $1\,mL \cdot min^{-1}$ using a Viscotek solvent/sample module VE2001, equipped with four Phenomenex columns (10^6, 10^5, 10^3 and 500 Å) and a Viscotek refractometer VE3580. The system was calibrated with polystyrene standards ranging from 500 to $10^6\,g \cdot mol^{-1}$.

Results and Discussion

Doxorubicin (Figure 1) is an important drug used for treatment of many tumors. As the use of this anticancer drug is associated with several side effects, drug delivery systems have been developed to minimize side effects and increase the drug efficacy,[22] including polymer beads used in chemoembolization procedures.[29,33,46] Therefore, the in situ incorporation of doxorubicin during VAc/MMA suspension copolymerizations can constitute an interesting strategy for production of chemoembolic agents. However, given the multifunctional nature of doxorubicin, chemical interactions with the growing polymer chains are likely to occur.

Preliminary solubility tests showed that doxorubicin is essentially insoluble in VAc, MMA and VAc/MMA mixtures, but completely soluble in aqueous solutions. Previous results[44,45] showed that multifunctional drugs with low monomer solubility tend to accumulate on the particle/water interface, causing significant morphological changes in the obtained particles, when added in situ in the monomer phase. Besides, addition of the drug does not affect the interfacial tension of the suspended droplets. Based on that, addition of doxorubicin was performed through the aqueous phase only. Optical micrographs (Figure 2) showed that it is possible to obtain spherical particles when the VAc/MMA copolymerization reactions are performed in presence of small amounts of doxorubicin ($0.12\,g \cdot L^{-1}$). However, it was also observed that the final particle surfaces were not homogeneous in the presence of doxorubicin. This was probably due to accumulation of doxorubicin on the particle/water interface, as the final copolymer material was obtained as a fine powder with a light reddish aspect, due to the deposit of doxorubicin (which presents intense red color in aqueous solution) at the particle surface. However, UV spectroscopic analyses (data not shown here) of the aqueous medium after the polymerization reaction did not allow for quantitative determination of the drug incorporation.

In order to improve the biocompatibility of the beads, the obtained copolymer resins were saponified to produce a thin PVA shell around the particles. Based on previous results,[40–42] saponification occurs mainly at the particle surface, so that the overall degree of saponification is expected to be lower than 10%, which is sufficient to guarantee the biocompatibility. As shown is Figure 2, saponification does not affect the overall particle morphology; however, saponification promotes modifications of the particle surface, leading to formation of a porous skin, as shown in Figure 3.

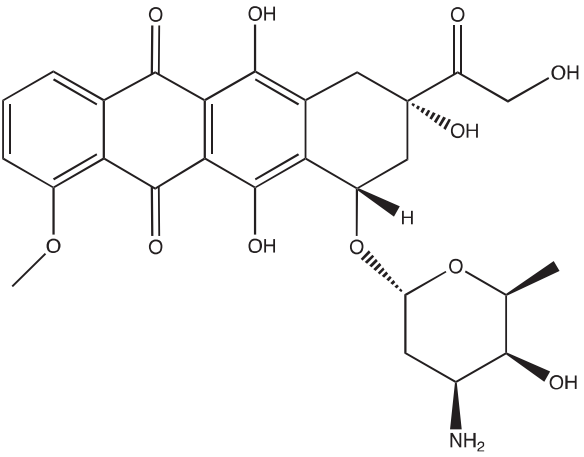

Figure 1.
Molecular structure of doxorubicin.

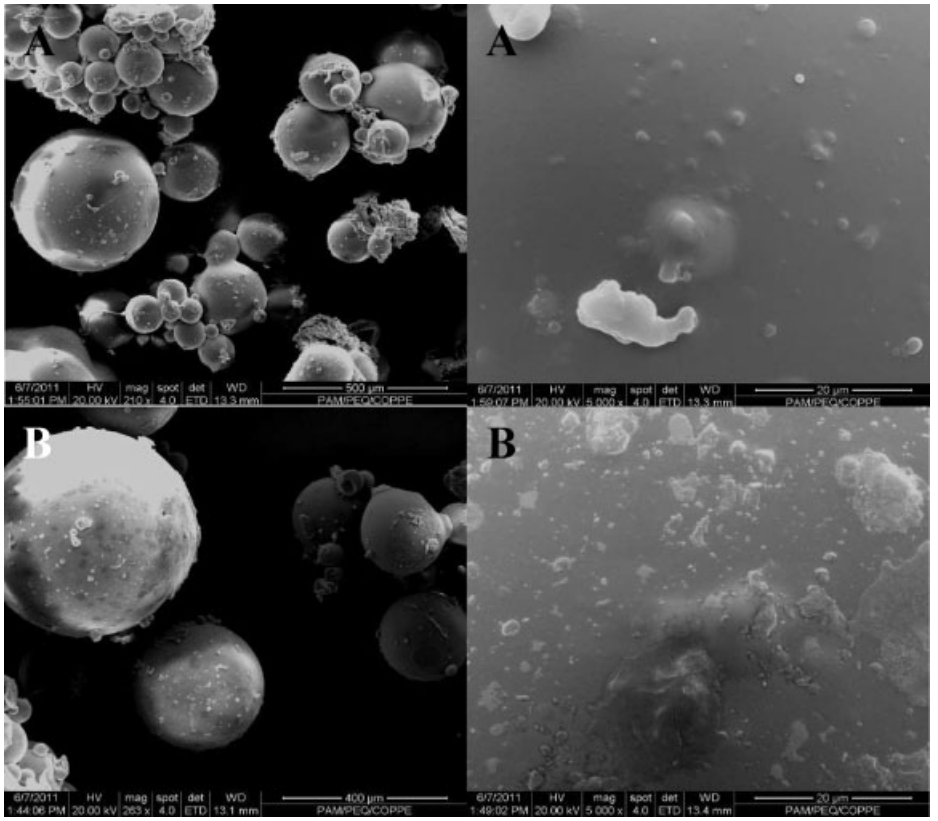

Figure 2.
VAc/MMA copolymer particles synthesized in the presence of doxorubicin during suspension polymerization. a) before saponification process; b) after saponification process.

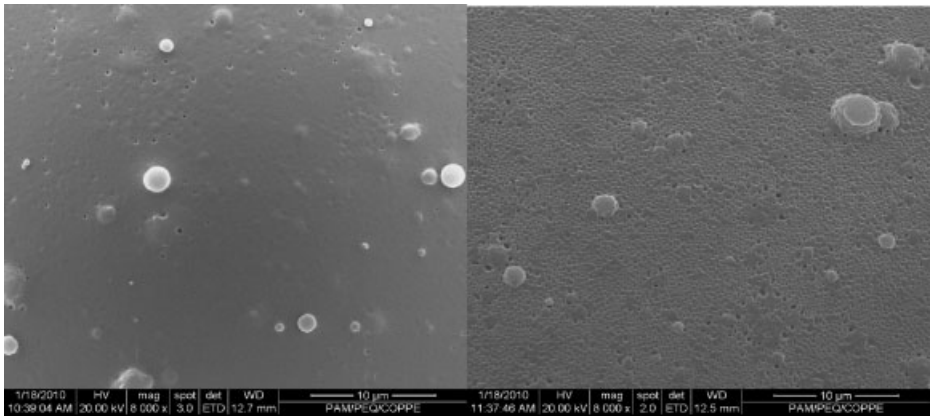

Figure 3.
VAc/MMA particle surface. a) before saponification process; b) after saponification process. Copolymer synthesized in absence of drug.

As reported previously,[44,45] formation of pores at the particle surface after saponification is probably related to the partial solubilization of the produced PVA chains. It had been observed previously that saponification can cause two different effects:[45] when the drug is added to the organic monomer phase, the drug gets entrapped into the polymer matrix and the formation of pores leads to higher rates of release of drug through the PVA *shell*; however, when the drug is added to the aqueous phase, the drug is distributed mainly on the particle surface and saponification leads to drug washing.

In order to verify if the drug had been partially incorporated into the polymer particles, ^{1}H-NMR analyses were performed, as shown in Figure 4. It is possible to identify the main characteristic resonance peaks of the random copolymer at $\delta = 4.8$ ppm (attributed to $-CHO-$ segments of the of PVAc) and at $\delta = 3.6$–3.8 ppm (attributed to $-COOCH_3$ segments of the PMMA). However, the characteristic signals of doxorubicin could not be detected, indicating the low incorporation of drug into the final beads. The signals observed at $\delta = 7.0$–8.0 ppm are related to the aromatic protons of the initiator (benzoyl peroxide).

Dynamic light scattering (DLS) analyses (Figure 5) showed that, when doxorubicin was added to the aqueous phase, the particle size distribution was in the range of 200–1000 μm. It was also observed that the saponification step shifted the particle size distribution towards smaller values (as it might already be expected because of PVA dissolution in the aqueous phase). The obtained size range was shifted towards much higher values, when compared to the sizes of the VAc/MMA copolymer particles produced in absence of drug (100–600 μm). As observed previously with amoxicillin, doxorubicin also affects the stability of the organic suspension, modifying the relative rates of particle coalescence and break-up and reducing the efficiency of the suspending agent. However, in all cases most particle sizes lie in the size range normally recommended for use in embolization procedures.[32] Fine tuning control of the final particle size could be achieved through manipulation of some of the process variables, such as the agitation speed.[47–49] Figure 5 shows clearly that addition of doxorubicin shifts the particle size distribution to higher values.

Monomer conversion was monitored when doxorubicin was added to the reaction medium during the VAc/MMA sus-

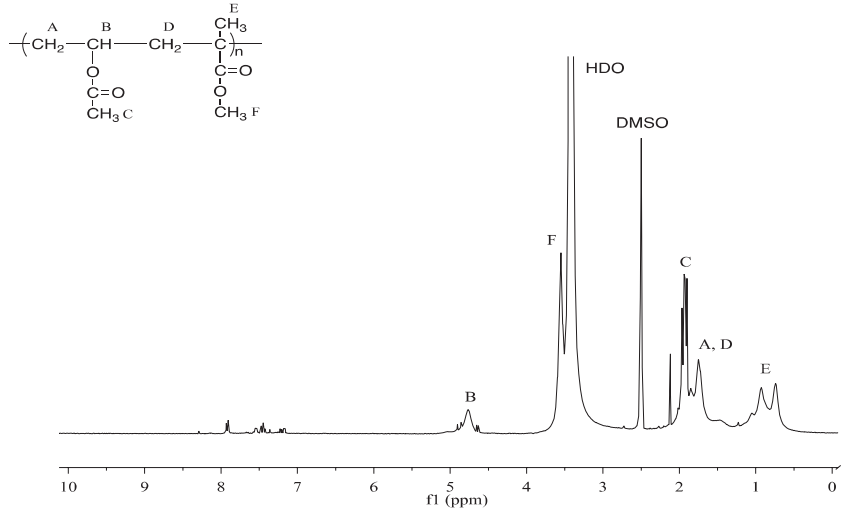

Figure 4.
^{1}H-NMR spectrum of VAc/MMA random copolymer synthesized in presence of doxorubicin.

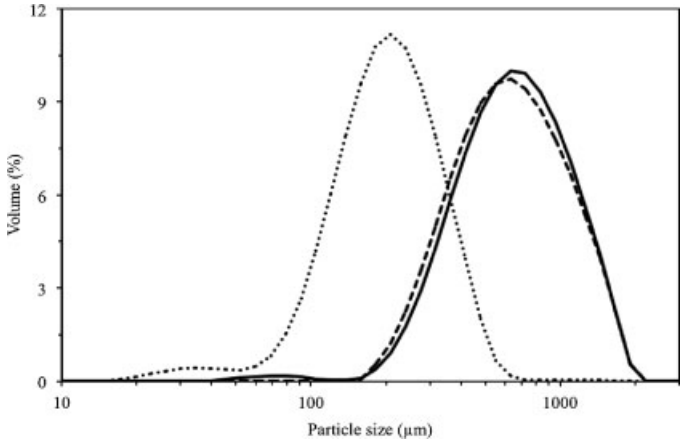

Figure 5.
Particle size distribution of VAc/MMA copolymer particles. (••••) PVAc-co-PMMA without drug; (––) PVAc-co-PMMA with drug; (----) PVAc-co-PMMA/PVA with drug.

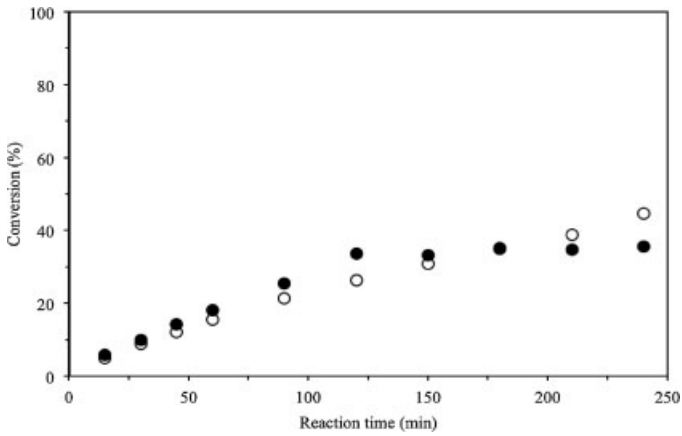

Figure 6.
Evolution of the global monomer conversion in absence (○) and in presence (•) of doxorubicin loaded in situ during the suspension copolymerization of VAc and MMA.

pension copolymerization. As shown in Figure 6, the copolymerization rate was affected by the presence of doxorubicin in the aqueous medium. Surprisingly, the inhibitory effect of the drug can only be observed clearly when the global monomer conversion reaches a certain critical value, as also observed previously for amoxicillin.[45] Therefore, the kinetic effects induced by the presence of doxorubicin can probably be related to diffusive limitations and the gel effect. This effect has yet to be explained, as it is not clear why the observed kinetic effects depend on the monomer conversion when a multifunctional drug molecule is added to the reaction system. A possible explanation is related to transfer to doxorubicin, as observed with amoxicillin. High conversions can only be achieved in presence of doxorubicin when the initiator concentration is high or when the reactor tempera-

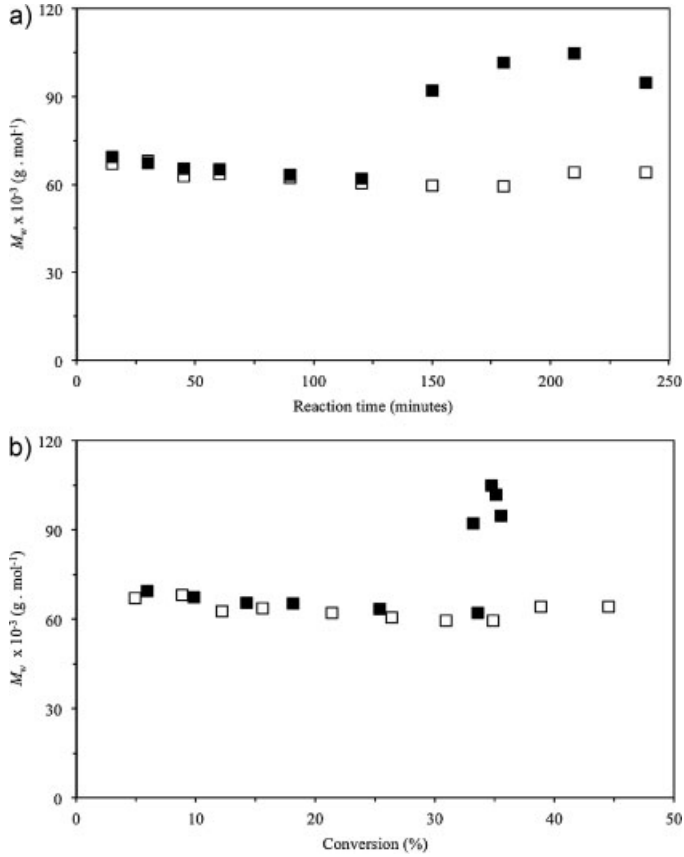

Figure 7.
Dynamic evolution of molecular weight averages (M_w) (a) and global monomer conversion (b) in absence (□) and in presence (■) of doxorubicin loaded in situ during the suspension copolymerization of VAc and MMA.

ture is increased. In all cases, a maximum conversion limit, as shown in Figure 6, seems to exist.

The *in situ* incorporation of doxorubicin also caused significant modifications of the dynamic trajectories of the molecular weight averages of the copolymer material during the polymerization, as shown in Figure 7. When the copolymerization reaction was carried out in absence of drug, M_w remained essentially constant throughout the reaction time. In presence of doxorubicin, though, M_w values remained constant until attainment of the limiting monomer conversion of ~35%, and suddenly increased to much higher values. Similar effects were also observed when a small amount of amoxicillin was used as a model drug, in a similar copolymerization reaction system, as described in previous works.[44,45] It was shown that the amoxicillin (a multifunctional drug) can affect the molecular weight in different manners, but generally leads to a simultaneously formation of shorter and long chains, due to chain transfer to the drug and to the polymer (crosslinking), respectively.

Conclusion

Copolymerizations of vinyl acetate and methyl methacrylate were performed in absence and in presence of doxorubicin in order to produce copolymer beads to be used as chemoembolic agents. At the

analyzed conditions, the in situ incorporation of doxorubicin promotes significant changes in the copolymer materials and copolymerization reactions. The morphology of the final particles changes in presence of doxorubicin and it seems that the drug is accumulated on the particle surface. The in situ addition of the drug can also affect the overall monomer conversion, as the drug exerts an inhibitory effect that becomes more evident after accumulation of polymer. The in situ addition of the drug can also affect the final molecular weight averages of the copolymers, as significant increase of the molecular weight averages can be observed after a certain period of time, indicating the possible crosslinking effect induced by the drug.

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