Synthesis of Nanoparticles Loaded with Tamoxifen by *in Situ* Miniemulsion RAFT Polymerization

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Summary: Tamoxifen (TXF) is a drug used as a hormonal agent for treatment of breast cancer. Due to its low solubility/bioavailability, the effectiveness of TXF can be improved when the drug is combined with drug delivery systems (DDS). For this reason, the *in situ* incorporation of TXF in polymer particles produced through miniemulsion polymerizations is studied here. Reactions were performed through standard free radical (FR) and RAFT polymerizations, using methyl methacrylate (MMA) as monomer and 2,2'-azobisisobutironitrila (AIBN) as initiator. It is shown that TXF can be incorporated successfully into the final polymer particles through miniemulsion polymerizations and that the presence of TXF in the reaction medium does not affect significantly the reaction rates, the particle size distribution and the molar mass distribution of the final polymer, even when the monomer feed contains 10 wt% of drug. Therefore, it is shown that DDS containing TXF can be produced by *in situ* miniemulsion FR and RAFT MMA polymerizations.

Keywords: methyl methacrylate (MMA); miniemulsion; nanoparticles; reversible addition fragmentation chain transfer (RAFT); tamoxifen

Introduction

Tamoxifen (TXF) is the only hormonal agent approved by the FDA (Food and Drug Administration) for breast cancer prevention, the second most frequent type of cancer in the world and the commonest type of cancer among women, accounting for 22% of new cases each year.[1] However, TXF belongs to the Biopharmaceutics Classification System II, presenting low water solubility and high permeability. The low water solubility compromises the TXF dissolution in living tissues, also compromising the absorption and bioavailability.[2] Therefore, a possible strategy to overcome these drawbacks is the preparation of drug delivery systems (DDS) that contain TXF, such as polymer nanoparticles (NP). The use of NPs for preparation of DDS can promote solubility, bioavailability, physicochemical and biological stability, accumulation and retention in tumor cells and decrease of tumor resistance mechanisms, reducing the side effects of medical treatments, especially when the DDS is targeted to reach specified cell populations.[3,4]

One of the techniques used most often to synthesize NPs is the controlled radical polymerization (CRP) performed in miniemulsion systems. Among the various existing CRP techniques, reversible addition fragmentation chain transfer (RAFT) polymerizations are usually regarded as more robust and appropriate for biomedical applications.[5–7] The standard RAFT polymerization is a free radical (FR) reaction performed in the presence of a chain

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transfer agent (CTA) that allows for proper control of the chain growth.[5,6] RAFT systems are very versatile because they usually allow for use of different monomers and for subsequent conjugation of CTA residues to biological or synthetic molecules.[7] As a consequence, NPs produced through RAFT miniemulsion polymerizations can be vectorized for special DDS applications.

The *in situ* incorporation of drugs during polymerization reactions is a well-known strategy to prepare a polymer-based DDS. The main advantage of this technique is the preparation of a doped polymer material in a single step, leading to easier, faster and less expensive production routes in the industrial scale. However, the presence of the drug in the reaction environment can affect the course of the polymerization process and the final properties of the obtained polymer resins, including the thermal behavior, the molecular weight distribution (MWD) and the particle size distribution (PSD) of particles prepared in heterogeneous systems.[8–10]

Based on the previous remarks, the main objective of the present study is to evaluate how miniemulsion polymerizations of methyl methacrylate (MMA), performed through conventional FR and RAFT polymerizations, and the properties of the obtained PMMA NP loaded with TXF are affected by the presence of TXF in the reaction medium. It is shown that TXF can be incorporated successfully into the final polymer particles through miniemulsion polymerizations and that the presence of TXF in the reaction medium does not affect significantly the reaction rates, the particle size distribution and the molar mass distribution of the final polymer, even when the monomer feed contains 10 wt% of drug.

Materials and Methods

Materials

Azobisisobutyronitrile (AIBN, 99%), sodium dodecyl sulphate (SDS, 98%), hydroquinone (99%), hexadecane (HD, 99%), methyl methacrylate (MMA, 99%), and sodium bicarbonate (Na_2CO_3, 98%) were purchased from Vetec Química Fina (Rio de Janeiro, Brazil). Tetrahydrofuran (THF, 99.9% HPLC grade), Methanol (99.9%, HPLC grade) and Glacial Acetic Acid (99.8%, HPLC grade) were purchased from Tedia (Rio de Janeiro, Brazil). 2-Cyanoprop-2-yl dithiobenzoate (CPDB, 99%), used as RAFT agent, and sodium 1-octanosulfonate (99%) were purchased from Sigma-Aldrich (Rio de Janeiro, Brazil). Tamoxifen free base (TXF free base, 98.7%) was obtained from Tamoxifen citrate, which was purchased from Pharma Nostra (Rio de Janeiro, Brazil) and its purity was determined through high performance liquid chromatography. The water used in all experiments was purified through a sequential three-step purification process, comprising distillation, demineralization and microfiltration. All other chemicals were used as received without further purification, except when indicated.

Methods

Miniemulsion Polymerization

The standard recipe employed to perform the miniemulsion polymerizations is described in Table 1. The aqueous phase was comprised of amouns of SDS (emulsifier) and sodium bicarbonate (buffering agent) specified in table in solubilized in water. The dispersed organic phase was prepared by mixing MMA, AIBN (initiator), HD (co-stabilizer), CPDB (for RAFT polymerizations) and TXF (when used) at the desired concentrations. The organic phase was then mixed with the aqueous phase for 10 min at 25 °C and finally sonicated (LB550, Labometric) for 10 min at 70% amplitude. The resulting emulsion was transferred to a 50 ml round-bottom flask purged with N_2 for 60 min at 15 °C. The polymerization reactions were conducted under magnetic stirring and samples were taken at specified sampling times for characterization.

Table 1.
Standard recipe for miniemulsion polymerization.

Material	Continuous Phase	Dispersed Phase	Amount (g)	Notes
Water	Water		40	
Surfactant	SDS		0.5	1% (*w/w*)[a]
Buffer Agent	Na_2CO_3		0.05	
Monomer		MMA	10	
Co-stabilizer		HD	0.3	0.65% (*w/w*)[b]
Initiator		AIBN	0.042[c]	[M]:[I] = 200:1
RAFT Agent		CPDB	0.23[c]	[M]:[RAFT] = 100:1
Drug		TXF free base	1[c]	10% (*w/w*) [b]

[a] Related to the continuous phase;
[b] Related to the dispersed phase;
[c] If used.

Monomer Conversion

Monomer conversion was determined gravimetrically. In a disposable aluminum vessel, about 1 g of sample was withdrawn from the reaction flask and mixed with a few drops of aqueous hydroquinone solution (1% *w/v*). Hydroquinone is a reagent that promotes the inhibition of polymerization by preventing the propagation of the chain, allowing to analyze the actual conversion at each time.

The mass of each sample was recorded immediately after the sampling process. After reaching the room temperature, all samples were dried under vacuum at 50 °C until constant weight.

Gel Permeation Chromatography (GPC)

GPC analyzes were performed in THF at 40 °C and flow rate of 1.0 ml/min, using a chromatograph (Viscoteck) equipped with a refractive index detector (Viscoteck, VE3580) and Phenomenex columns. The system was calibrated with polystyrene standards with average molecular weights ranging from 500 to 10^6 g/mol. Theoretical values of the number average molecular weights ($Mn_{,te}$) of polymer samples prepared in presence of the RAFT agent were calculated with Equation 1, where α is the conversion of MMA.

$$M_{n,t,\theta} = \left(\frac{[M]_0 \times MW_{mon} \times \alpha}{[RAFT]_0}\right) + MW_{RAFT} \quad (1)$$

Particle Size

Particle size measurements were performed at 25 °C through light scattering device (Malvern Zetasizer Nano ZS), equipped with a laser source of 633 nm and a photodiode detector series. The original emulsion samples were diluted 100 times with pure water, which was previously filtrated through membranes with pores of 0.45 μm.

Differential Scanning Calorimetry (DSC)

Samples of about 3 mg were subjected to heating from 25 to 500 °C with a heating rate of 10 °C/min in aluminum crucible spouts. Nitrogen was used as the carrier gas at a flow rate of 80 ml/min and an empty aluminum pan was used as reference. The thermograms obtained for nanoparticle samples were compared with those obtained for samples of the pure polymer and of the pure drug.

TXF Encapsulation Efficiency

Polymer samples of 50 mg were added to 5 ml of methanol and subjected to ultrasound treatment (TEDIA, 99,85%) for 30 minutes and filtrated through a membrane with pores of 0.45 μm. The filtrate was injected directly into a high performance liquid chromatograph (HPLC, Shimadzu LC 10 ADvp) equipped with a CN column (Shimadzu) of 250 mm x 4.6 mm, 5.0 μm. The mobile phase used was a methanol solution containing, in each liter, 320 mL of

Water, 2 mL of glacial acetic acid and 1.08 g of sodium 1-octanesulfonate. The mobile phase was fed at 1.0 ml/min and 20 μl of the sample were injected for analyses. Concentration readings were performed by light absorption at 254 nm with the help of a calibration curve built with pure TXF.

Results and Discussion

MMA miniemulsion polymerization reactions were carried out at 80 °C by conventional FR and RAFT polymerizations, using AIBN as initiator in the presence and absence of TXF. Figure 1 shows the evolution of monomer conversion as a function of time in both reaction systems. As one can see, conventional FR reactions are relatively fast, reaching conversions that are close to 90% after 30 minutes, while RAFT reactions are much slower, reaching conversions around 90% only after 240 minutes. The decrease of the reaction rates in RAFT polymerizations is well understood and is related to the CTA activity, which keeps the growing polymer chain in a dormant state for long periods of time.[5]

Figure 2 shows how the number average molecular weights (Mn) and polydispersities (Mw/Mn) change with monomer conversion in both analyzed reaction systems. Polydispersities and Mn values do not seem to be affected by the presence of the drug in the reaction environment in both polymerization schemes. In conventional FR reactions, polydispersities were larger than 1.5 throughout the reaction course, as it might already be expected. Besides, Mn values were higher in the early stages of the reaction, decreasing gradually with conversion due to monomer consumption.[5] The

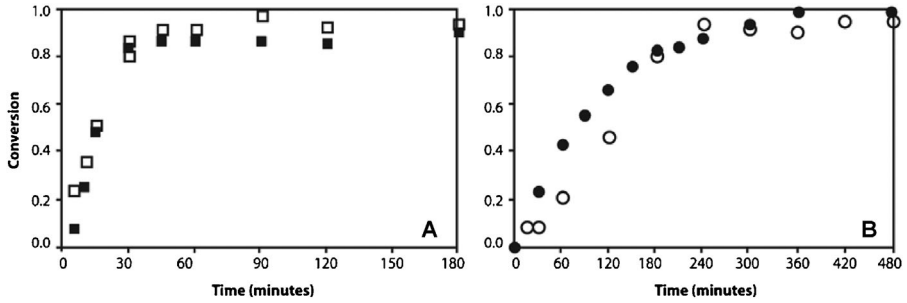

Figure 1.
Conversion vs *Time* for miniemulsion MMA polymerizations in (□; ○) absence and in (■; ●) presence of TXF based on recipes presented in Table 1 for (a) FR and (b) RAFT polymerizations.

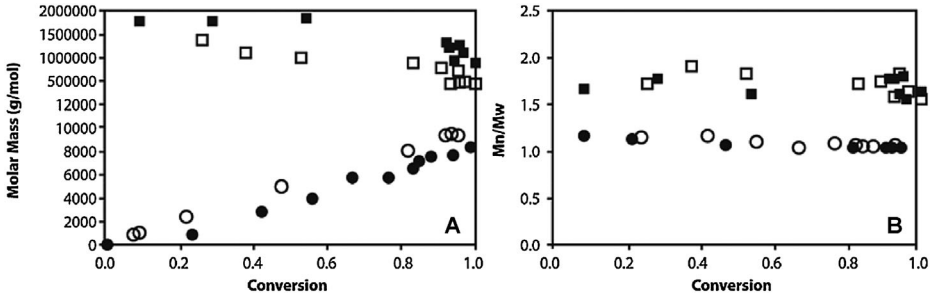

Figure 2.
Mn and M_w/M_n vs *Conversion* for miniemulsion RAFT MMA polymerizations performed in (□; ○) absence and in (■; ●) presence of TXF, based on recipes presented in Table 1 for (□, ■) FR and (○, ●) RAFT polymerizations.

highest polydispersity observed for RAFT reactions was equal to 1.2 at low (8%) monomer conversions, decreasing to 1.1 at conversions of 99%. Additionally, Mn values increased continuously and linearly with monomer conversion until attainment of the maximum expected theoretical value of 10,000 g/mol, calculated with Equation 1. This linear Mn growth and low polydispersities demonstrate that the MMA RAFT polymerization occurred in a controlled manner and confirm the *"living"* character of the reaction.

Figure 3 shows the MWD of polymer samples produced with the RAFT CTA in the absence and presence of TXF. It is possible to observe the shifting of the MWD towards higher molecular weight values when monomer conversion increases, as also shown in Figure 2. It must be noted that the MWDs were not affected significantly by the presence of TXF.

Miniemulsion FR and RAFT MMA polymerizations lead to formation of stable latexes, both in absence and presence of TXF. Average particle sizes are presented in Figure 4. It is shown that TXF does not affect the average particle sizes, although particle sizes are larger for RAFT polymerizations because of the much lower reaction rates. In all cases, the size distributions were monomodal (Figure 5) and the PSD polydispersities were low, showing that the employed homogenization technique was satisfactory and that particle nucleation was efficient. As the final particle size

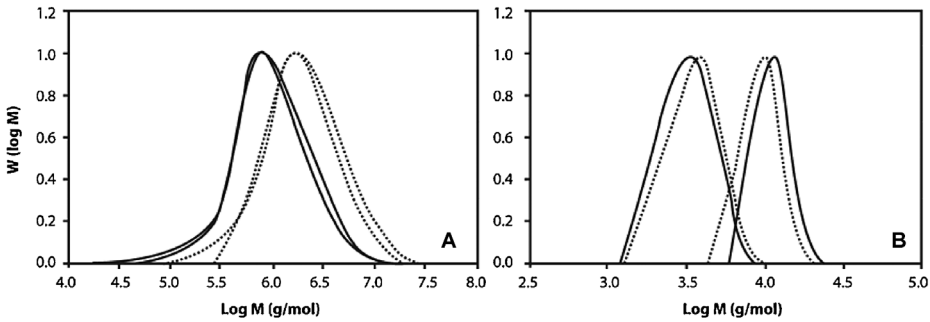

Figure 3.
MWD of polymer samples produced through miniemulsion RAFT MMA polymerizations (a) in absence and (b) in presence of TXF. The reaction times are (a) 10 and 180 minutes; and (b) 30 and 480 minutes respectively.

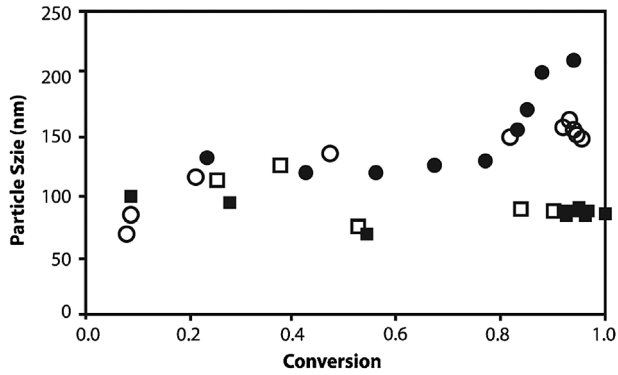

Figure 4.
Particle Size vs. Conversion for miniemulsion RAFT MMA polymerizations performed in (□; ○) absence and in (■; ●) presence of TXF, based on recipes presented in Table 1 for (□, ■) FR and (○, ●) RAFT polymerizations.

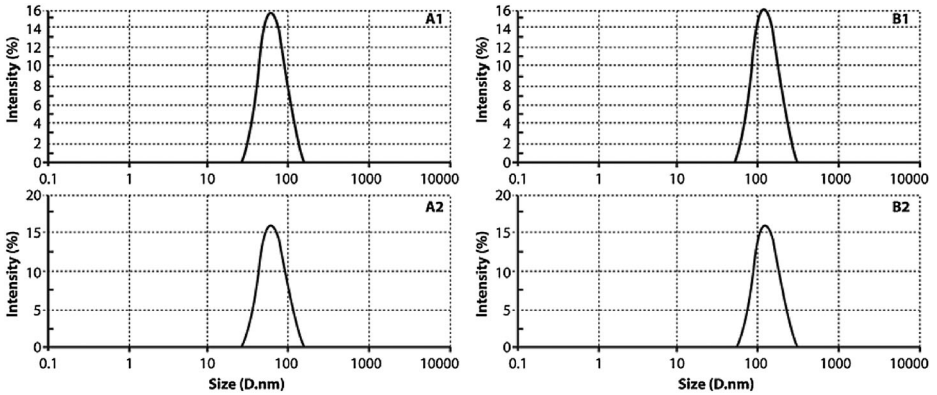

Figure 5.
Intensity vs. size for miniemulsion RAFT MMA polymerizations performed in (**1**) absence and in (**2**) presence of TXF, based on recipes presented in Table 1 for (**A**) FR and (**B**) RAFT polymerizations.

ranges were narrow and were not affected by the presence of TXF, it is possible to expect the uniform rate of drug release if nanoparticles are used as DDS.

Tests performed to evaluate the efficiency of the TXF encapsulation showed that $91.0 \pm 1.8\%$ and $81.2 \pm 2.1\%$ of the initial amounts of TXF added to the reaction were incorporated into the polymer particles obtained through RAFT and conventional FR reactions, respectively. DSC results presented in Figure 6 confirm that TXF did not form a distinct phase in the NP, but dissolved in the polymer matrix, as the thermograms of NPs prepared in the presence of TXF did not present the characteristic endothermic transition temperature of the drug.

Conclusion

Miniemulsion MMA polymerizations were carried out in absence and presence of tamoxifen (TXF). Reactions were performed through standard free radical (FR) and RAFT polymerizations and it was shown that TXF can be incorporated successfully into the final polymer particles *in situ*, with encapsulation efficiencies around 80% and 90% for FR and RAFT polymerizations, respectively. It was also

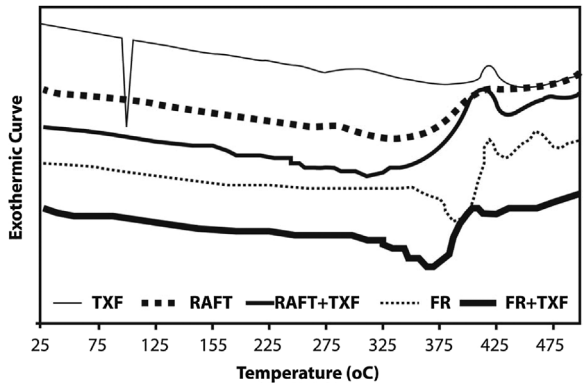

Figure 6.
DSC thermograms of polymer samples obtained in absence and presence of TXF through miniemulsion FR and RAFT MMA polymerizations.

shown that the presence of TXF in the reaction medium does not affect significantly the reaction rates, the particle size distribution and the molar mass distribution of the final polymer, even when the monomer feed contained 10 wt% of drug. The latex obtained in all reactions was stable and free of clots. Therefore, it was shown that drug delivery systems containing TXF can be developed by *in situ* miniemulsion FR and RAFT MMA polymerizations.

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