http://dx.doi.org/10.4322/polimeros.2014.050

Effect of Tamoxifen in RAFT Miniemulsion Polymerization During the Synthesis of Polymer Nanoparticles

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Abstract: Tamoxifen (TXF) is currently the only hormonal agent used for treatment of breast cancer. Although very effective, TXF presents low solubility in water, which affects its absorption and bioavailability. A common strategy to overcome this barrier is the formulation of a drug delivery system (DDS) in order to increase the drug stability and improve the treatment effectiveness. Reversible addition-fragmentation chain transfer (RAFT) polymerization is the most versatile method of controlled/living radical polymerization (CLRP), allowing for synthesis of well-defined polymers and being adapted to a wide range of polymerization systems. Miniemulsion polymerization is a dispersed system that is commonly used to prepare nanoparticles (NP) with 50 to 500 nm of diameter. The aim of this work was to evaluate the effect of the *in situ* incorporation of TXF during miniemulsion conventional and RAFT polymerizations, using methyl methacrylate (MMA) as monomer. Although the *in situ* addition of TXF promoted a slight reduction of the reaction rate, it did not affect the final particle size distribution of the latex or the molecular weight control exerted by the RAFT agent. The obtained results suggest that *in situ* incorporation of TXF during the synthesis of polymer NP via RAFT polymerization allows for production of a polymer DDS for different uses, such as the breast cancer treatment.

Keywords: *Nanoparticles, drug delivery systems, RAFT polymerization, tamoxifen, methyl methacrylate.*

Introduction

Breast cancer is the second leading cause of cancer deaths nowadays and is the commonest cancer among women[1]. Many types of breast cancer present estrogen receptors, so that growth of these tumors can be stimulated by estrogen. For this reason, breast cancer treatments normally attempt to reduce estrogen levels with help of antiestrogens and progestational agents[2]. For over a quarter of a century, tamoxifen (TXF) has been used for treatment of estrogen receptor-positive breast cancers by competing with estrogen for binding with estrogen receptors in breast cancer cells[3,4].

TXF is employed mainly for the long-term prophylactic therapy in high-risk and post-menopausal women[3]. As TXF therapy can be regarded as chronic (during 3 to 5 years), oral delivery is the preferred route for TXF administration. The poor TXF solubility in aqueous media can be overcome through preparation of a derived salt, the TXF citrate (TXFc). Commercially, this drug is available only as tablets and oral solutions containing TXFc for daily doses of 10 or 20 mg. However, TXF citrate also presents poor oral bioavailability (20-30%) due to precipitation as a free base in the acidic stomach environment and also due to the intense hepatic and intestinal first pass metabolism[5]. Therefore, although the use of TXF constitutes a usual clinical choice for treatment during advanced and metastatic stages of breast cancer, TXF treatment is also subject to large inter-subject variability and to several dose and concentration dependent side effects[6,7]. The most significant side effect is related to the estrogenic effect in the uterus, which increases the risk of endometrial cancer and development of drug resistance, possibly leading to further progression of the tumor[8-10]. Other side effects include liver cancer, pulmonary embolism, venous thrombosis and ocular side effects, including retinopathy and corneal opacities[11]. These unwanted side effects and the many barriers for the effective administration of the drug requires the targeted delivery to the tumor site and the consequent enhanced drug uptake by the tumor cells[12,13].

Drug delivery systems (DDS) hold great potential for efficient targeting of many types of cells. Particularly, drug-loaded nanoparticles (NP) can be designed and engineered to pass through the fenestrations of the leaky cancer endothelium and reach the tumor cells more efficiently[14]. For instance, it has been demonstrated that use of NPs doped with TXF can lead to increase of drug concentration in tumors through enhanced retention and permeability effects (RPE)[15-19]. Therefore, in order to overcome undesirable side effects caused by TXF and to enhance targeting specificity, TXF can be encapsulated in polymer NPs. This can also allow for reduction of drug resistance problems and facilitation of drug transport across natural barriers.

Polymer NPs can be prepared through free radical miniemulsion polymerizations[20]. In the conventional free radical polymerization, radicalar species are formed by the homolytic cision of a covalent bound at

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the initiator molecule (Figure 1). The active radicalar species attack the unsaturated vinyl bonds of monomer molecules almost immediately, leading to formation of monomeric free radicals that initiate the polymerization. The polymer product is formed through the successive addition (propagation) of monomer molecules to the active free radical center. In theory, propagation reactions can continue until monomer exhaustion, although this event is unlikely because the chain growth is interrupted by termination reactions[21]. The premature termination of chain growth can prevent the proper control of average molecular weights and molecular weight distributions of the obtained polymer material, affecting the mechanical properties and, consequently, the performances of the final product. This effect can limit the use of conventional free radical polymerizations for syntheses of polymers with well-defined molecular architectures, copolymer compositions and low polydispersities (PDI)[22].

Controlled living radical polymerizations (CLRP) constitute useful alternatives for preparation of polymer materials with well-defined molecular architectures. Essentially, CLRP are radical polymerizations with very low rates of radical termination, allowing for more uniform chain growth (Figure 2). In this case, most radical chains grow simultaneously, leading to products with low PDIs and narrow molecular weight distributions. Moreover, the number-average molecular weight of the obtained polymer material grows linearly with monomer conversion[23-27]. CLRPs can also allow for control of chain compositions during semibatch reactions, through manipulation of the monomer composition in the reaction medium, leading to preparation of copolymer materials with very peculiar molecular architectures[28]. Among the several known CLRP techniques, reversible addition-fragmentation chain transfer polymerization (RAFT), atom transfer polymerization (ATRP) and nitroxide mediated polymerization (NMP) have received more attention in the literature[23].

RAFT polymerizations (Figure 2) present several competitive advantages when compared to other CLRP techniques, including the possible use of different solvents, monomers and polymerization systems[24-27] and the versatility to produce polymers with well-defined complex architectures[28]. In RAFT polymerizations, a chain transfer agent with the general structure ZC(=S) SR, where Z is an activating group and R is a transferred chemical group, is used to control the chain growth. The thio-carbonyl-thio terminal group present at the final polymer chains can be easily converted to a thiol group for posterior chemical modifications of the polymer material[25,29]. For instance, thiol groups can be used for bioconjugation and development of biomedical applications[29-31].

The *in-situ* incorporation of drugs during polymerization reactions constitutes a well-known strategy to prepare a polymer DDS. The main advantage of this technique is the production of doped polymer particles in a single step, allowing for development of simpler industrial processes. However, the *in-situ* incorporation of drugs can affect the course of the polymerization process and the final properties of the obtained polymers, leading to modification of the thermal behavior, molecular weight distributions and particle size distributions (in the case of heterogeneous reactions)[32-35].

Based on the previous paragraphs, in the present study miniemulsion polymerizations of methyl methacrylate (MMA) are performed in the presence of TXFc and a RAFT agent (2-Cyanoprop-2-yl dithiobenzoate, CPDB) in order to produce poly(methyl methacrylate) (PMMA) NPs loaded with the anti-cancer drug. To the best of our knowledge, this is the first time that the synthesis of polymer NPs doped with TXF through RAFT miniemulsion polymerization is reported.

Experimental

Materials

Benzoyl peroxide (BPO, 97%), dodecyl sodium sulphate (SDS, 98%), hydroquinone (99%), hexadecane (HD, 99%), methyl methacrylate (MMA, 99.5%), and sodium bicarbonate (Na_2CO_3, 98%) were purchased from Vetec Química Fina (Rio de Janeiro, Brazil).

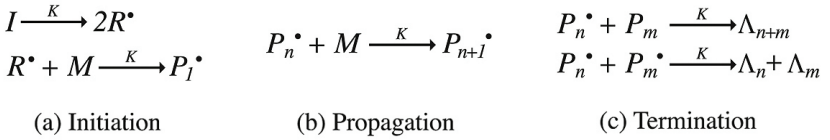

Figure 1. Classical free radical polymerization mechanism. I = initiator; M = monomer; R● = any radicalar species.

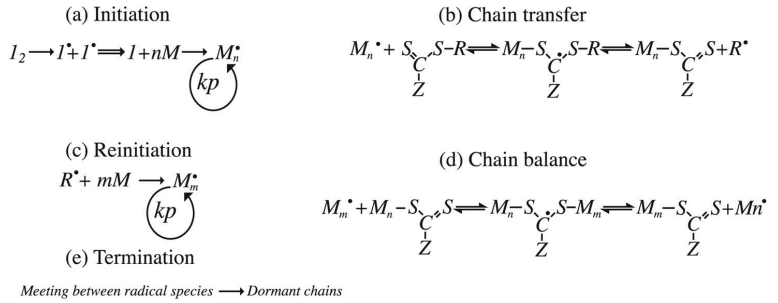

Figure 2. Standard RAFT polymerization mechanism. I = initiator; M = monomer, R = transferred group; Z = activation group. Some species have been edited for brevity.

Tetrahydrofuran HPLC grade (THF, 99.9%) was purchase from Tedia (Rio de Janeiro, Brazil). 2-Cyanoprop-2-yl dithiobenzoate (CPDB, 99%) was purchased from Sigma-Aldrich (Rio de Janeiro, Brazil) and used as RAFT agent. Tamoxifen citrate (TXFc, 99.9%) 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 by a sequential three-step process (distillation, demineralization, and microfiltration). All other chemicals were used as received without further purification, except when indicated.

Methods5

Miiniemulsion polymerization

The basic miniemulsion recipe is shown in Table 1. First, a solution of SDS in water was prepared at room temperature, adding a fixed amount of sodium bicarbonate as buffer agent. In a second flask, the organic phase was prepared by dissolving MMA, BPO, HD, CPDB (for RAFT polymerizations) and TXFc (if used). The organic (dispersed) phase was mixed with the continuous (aqueous) phase for 10 min at 25 °C, and then ultrasonicated (LB550, Labometric) for 10 min at 70% of amplitude in an ice bath. In order to improve the emulsification process, the miniemulsion was kept under continuous magnetic stirring during ultrasonication. The resulting miniemulsion was transferred to a 50 ml rounded-bottom flask and degassed with N_2 for 60 min at 15 °C. Polymerization runs were carried out at 90 °C under continuous magnetic stirring. During the reaction, samples were taken at predetermined times for characterization.

Conversion

Monomer conversion was determined gravimetrically. In a disposable aluminum vessel, approximately 2 g of sample were taken from the reactor and mixed with few drops of an aqueous hydroquinone solution (1 wt.%). The weight of each sample was recorded immediately after sampling. After reaching the room temperature, all samples were dried at 50 °C until constant weight in a drying oven.

Gel Permeation Chromatography (GPC)

GPC analyses were performed in THF at 40 °C and with flow rate of 1 ml/min, using a Viscoteck system comprising a VE2001 pump-injetctor module, a column oven, and a VE3580 refractive-index. The system was equipped with a Phenomenex 5.0 mm bead-size guard column (50 × 7.5 mm^2), followed by two linear PL gel columns (Mixed C and 500 Å) calibrated with polystyrene standards ranging from 500 to 10^6 g/mol.

For polymer samples synthesized in presence of the RAFT agent, theoretical number-average molecular weights ($M_{n,th}$) were calculated based on Equation 1, were α denotes the fractional conversion of MMA; $[M]_0$ and $[RAFT]_0$ denote the initial concentrations of MMA and RAFT agent; and MW_{mon} and MW_{RAFT} denote the molecular weight of the RAFT agent.

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

Dynamic light scattering

Dynamic light scattering measurements were conducted with a Malvern Zetasizer Nano ZS instrument equipped with a 4 mW He-Ne laser operating at λ = 633 nm and with an avalanche photodiode detector with high quantum efficiency. Before the measurements, the original latex samples were diluted (1 drop of latex in 2 ml of water, previously filtrated in filters of 0.45 μm) for adjustment of the light strength. All measurements were conducted at 25 °C.

Results and Discussion

MMA polymerization reactions were conducted in miniemulsion by conventional and RAFT free radical polymerizations at 90 °C and using the BPO as the main source of free radicals. Further, in order to prepare drug-loaded NPs and study the effects of the *in-situ* incorporation of TXFc in the polymerization reactions, reactions were conducted in absence and presence of TXFc. Figure 3 shows monomer conversions obtained in the presence and absence of TXFc via conventional and RAFT polymerization reactions. As one can observe, conventional reactions were relatively fast, reaching monomer conversions around 90% after 30 minutes of reaction and values close to 99% after 180 minutes. Although both conventional reactions led to similar monomer conversion profiles, addition of TXFc apparently caused a slight inhibitory effect on the reaction kinetics. One can also observe in Figure 3 that reactions conducted in presence of the RAFT agent were much slower than the conventional reactions, as it might already

Table 1. Standard recipe for miniemulsion polymerizations.

Material	Continuous Phase	Dispersed Phase	Amount (g)	Notes
Water	Water		40	
Surfactant	SDS		0.5	1 (wt. %)[a]
Buffer Agent	Na_2CO_3		0.05	
Monomer		MMA	10	
Co-stabilizer		HD	0.3	0.65 (wt. %)[b]
Initiator		BPO	0.12	[M]:[I] = 200:1
RAFR Agent		CPDB	0.23[c]	[M]:[RAFT] = 100:1
Drug		TXFc	0.65[b,c]	

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

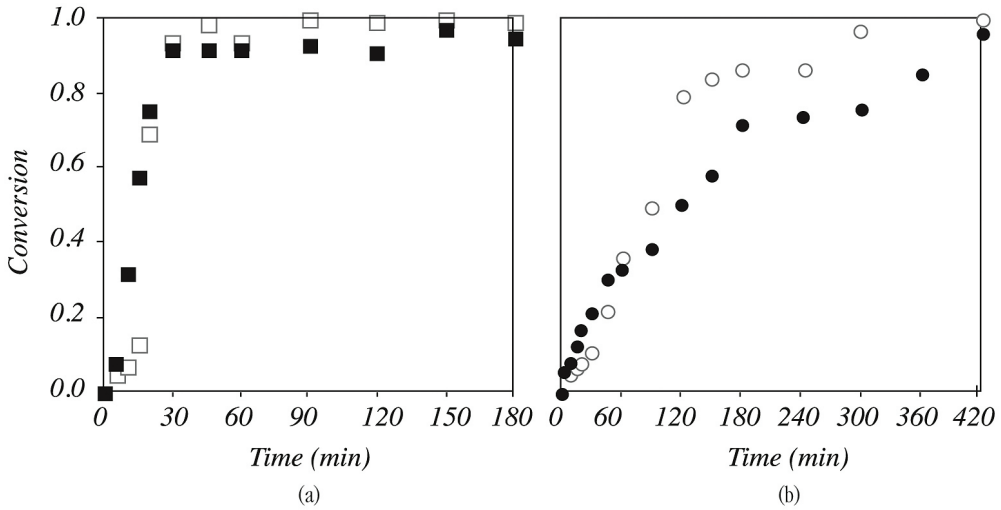

Figure 3. Monomer conversion profiles for miniemulsion polymerization of MMA in (□; ○) absence and in (■; ●) presence of TXFc, based on recipes presented in Table 1. (a) conventional polymerization; (b) RAFT polymerization.

be expected. The decrease of the reaction rates occurs because the RAFT agent keeps the growing polymer chains in a dormant stage for long periods of time[24-27]. It is possible to observe that the addition TXFc promoted a pronounced reduction of the reaction rates in presence of the RAFT agent, although final monomer conversions were close to 99% after 420 minutes of reaction. The inhibitory effect of drugs on the reaction rates of free radical polymerizations is due to the existence of multiple functional groups in the drug molecule that are capable to interact with the growing radicals, including hydroxyl, carbonyl, carboxylic, amine, among other chemical groups.

Figure 4 shows the variation of Mn and PDI of polymer samples as functions of monomer conversions. PDIs and Mns were not affected by the presence of the drug in both types of polymerization. In the conventional free-radical reactions, PDIs were higher than 2.0, as it might already be expected, as reaction rates are controlled by termination[20]. Mn values were higher during the first stages of polymerization due to depletion of monomer along the reaction run, as also expected for this type of mechanism[20]. On the other hand, the maximum PDI observed for reactions performed in presence of the RAFT agent was equal to 1.2 for monomer conversion of 8%, decreasing to values around 1.1 for monomer conversions of 99%. Mn values of polymer samples increased linearly with monomer conversion in presence of the RAFT agent, reaching the expected theoretical value of 10 000 g/mol, calculated according to Equation 1. The observed linear growth and the low PDI values demonstrate that the MMA polymerization via RAFT mechanism occurred in a controlled manner.

Figure 5 shows the molecular weight distributions of polymer samples prepared through miniemulsion polymerizations in presence of the RAFT agent in the absence and presence of TXFc. Independent from addition of the drug, it is possible to observe the considerable displacement of molecular weight distributions as monomer conversion increases, which is a characteristic

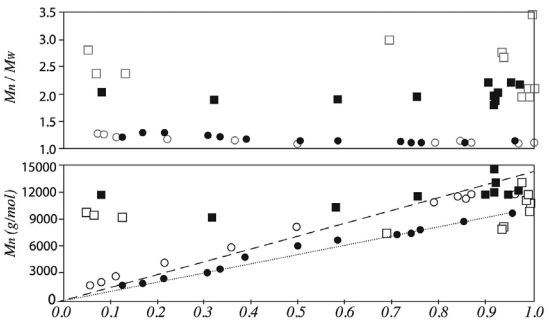

Figure 4. Mn and PDI values for miniemulsion RAFT polymerizations of MMA performed in (□; ○) absence and in (■; ●) presence of TXFc, based on recipes presented in Table 1. (□, ■) conventional polymerization and (○, ●) RAFT polymerization.

behavior of CLRP systems, demonstrating the "living" character of the RAFT reactions[36]. One can also observe that molecular weight distributions become narrower as the monomer conversion increases, due to the more uniform growth of polymer chains.

The miniemulsion polymerization reactions of MMA performed through conventional and RAFT free-radical mechanisms, both in the absence and presence of drug, resulted in stable latexes (phase separation could not be observed after one month of storage at rest) without formation of coagula. The obtained average particle sizes are shown in Figure 6, indicating that particle sizes were higher for RAFT polymerizations because of the much longer reaction times, which allow for particle growth through coalescence and diffusion degradation (Ostvald ripening). All size distributions presented low PDI values (around 1.05) and the characteristic monomodal behavior, demonstrating that homogenization was satisfactory. As all particle size distributions were narrow and were not affected by the presence of the drug, one can expect uniform rates of drug release in applications that use the obtained NPs for development of DDS[37].

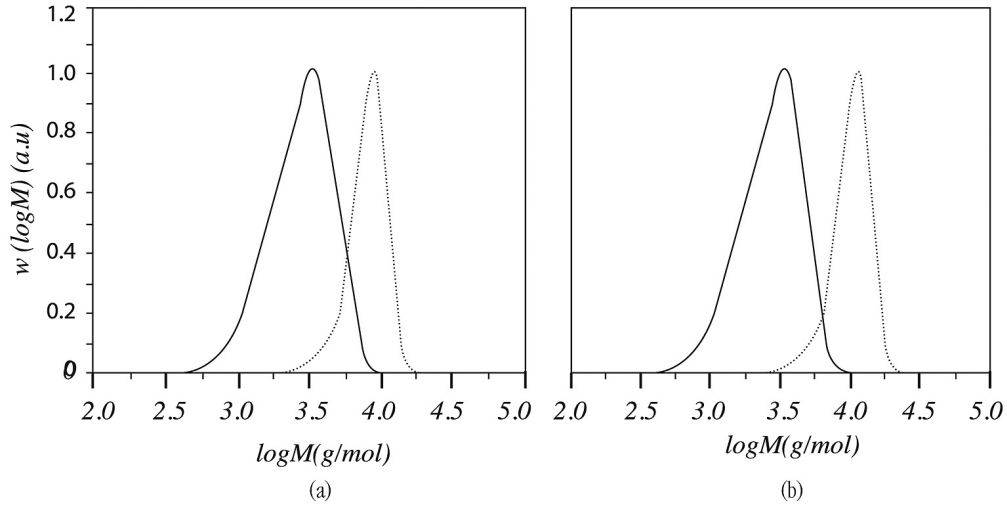

Figure 5. Molecular weight distributions of polymer samples prepared through miniemulsion RAFT polymerizations of MMA (a) in absence and (b) in presence of TXFc, based on recipes presented in Table 1. Average molecular weights increase from left to right. Monomer conversion values were equal to (a) 22 and 99% (b) 21 and 96%, respectively.

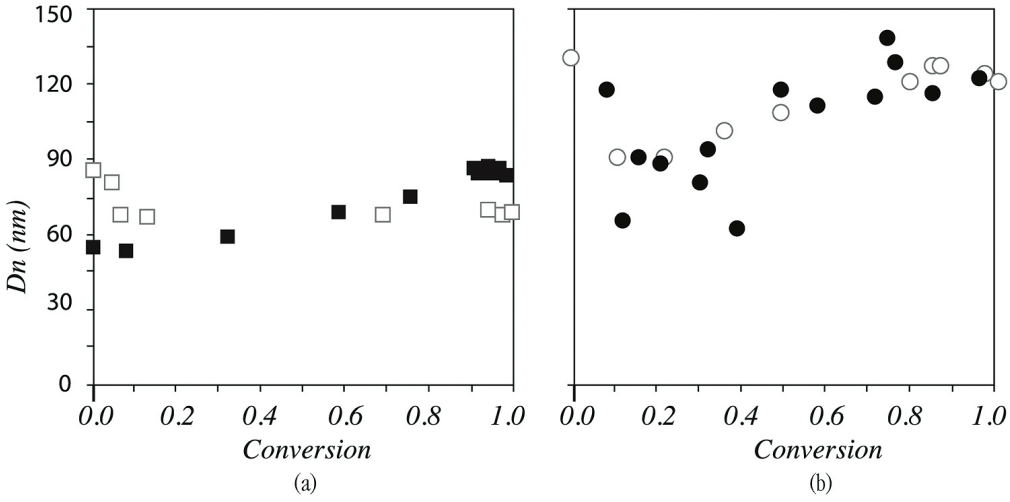

Figure 6. Average particle sizes of polymer particles produced through miniemulsion RAFT polymerization of MMA in (□; ○) absence and in (■; ●) presence of TXFc, based on recipes presented in Table 1. (□, ■) conventional polymerization and (○, ●) RAFT polymerization.

Conclusion

RAFT and conventional miniemulsion polymerization reactions of methyl methacrylate (MMA) were conducted in absence and presence of tamoxifen citrate (TXFc). The *in-situ* addition of 0.65 (wt. %) of TXFc in the dispersed organic phase did not cause any significant change of the reaction kinetics or properties of the obtained polymers, although a slight inhibitory affect could be assigned to the presence of the drug. All RAFT reactions presented the characteristic linear increase of the number-average molecular weight with monomer conversion and allowed for production of polymer materials with low polydispersities (≤ 1.1), confirming the controlled nature of the analyzed RAFT reactions even in presence of the drug. The proposed formulations led to production of stable latexes, without formation of coagula and narrow particle size distributions, allowing for future preparation of drug delivery systems.

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Received: 01/30/13
Revised: 08/28/13
Accepted: 10/21/13