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Preparation and Cytotoxicity of Poly(Methyl Methacrylate) Nanoparticles for Drug Encapsulation

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Summary: This work aimed to produce poly(methyl methacrylate) nanoparticles for use in drug encapsulation. The polymer nanoparticles were produced using miniemulsion polymerization technique. Monomer miniemulsion showed moderate stability and polymer average particle size was about 90 nm. PMMA nanoparticles were tested for toxicity in human leukemic cell strain K562 and they did not show any adverse effect on cell viability. Therefore, poly(methyl methacrylate) nanoparticles are suitable to encapsulate antitumor agents.

Keywords: biopolymers; emulsion polymerization; microencapsulation; nanoparticles; toxicity

Introduction

The development of nanoparticles in the biomedical area has the potential to greatly improve the diagnosis and treatment of many diseases, because it allows drug targeting and controlled release to a particular subset of cells. For the development of engineered nanoparticles is necessary to understand the importance of nanoparticle characteristics such as size, shape, surface properties and interactions with subsets of cells to create new opportunities for the development of nanoparticles for therapeutic applications.[1]

In the last two decades, therapies based on nanoparticles were successfully introduced for the treatment of cancer and infectious diseases.[1–2] Some therapies can use the properties of nanomaterials to target a drug at a specific site of action, to improve their apparent solubility, to increase their half-life and to reduce their immunogenicity, amplifying the effectiveness of the drug.[1]

Nanoencapsulation of drugs with biocompatible polymers can bring the advantages of the nanotechnology to pharmaceutical products.[3] Chemical engineering and the pharmaceutical industry have emphasized the development of such encapsulated products in order to improve the selectivity or minimize adverse effects associated with many drugs.[4–5]

Some important biopharmaceutical aspects, such as water solubility and pKa, strongly influence the administration route, therapeutic response and adverse effects; therefore these pharmaceutical parameters have a decisive influence in the design of drug delivery systems. Amphotericin B, for example, is an anti-fungal drug (also recommended for the treatment of leishmaniasis) that should be administered parenterally because it is highly hydrophobic and poorly absorbed if administered orally.[6] Additionally, when amphotericin B is administered intravenously in a mixed micellar solution, a range of adverse effects

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such as hemolysis and nephrotoxicity are observed, despite its efficacy.[5,7]

Nevertheless, parenteral administration of amphotericin B in a liposomal formulation not only improves the therapeutic efficiency but also minimizes the adverse effects to a considerable extent.[8] This example clearly shows that a well chosen drug delivery system can dramatically improve the performance of the drug. Given these issues, it was sought to focus on a polymer system that could act as viable candidates to encapsulate hydrophobic drugs, at a low cost and a particle size in the nanometer range.

Poly(methyl-methacrylate) (PMMA) particles are systems with a good biocompatibility that can be produced in emulsion, miniemulsion and suspension polymerization processes. Some researchers have successfully used PMMA nanoparticles in the treatment diseases such as Duchenne muscular dystrophy, cancer, etc.[15–19] Araujo *et al.* 1999 studied the oral administration of PMMA nanoparticles in order to evaluate nanoparticles uptake from rat gastrointestinal tract.[14]

In this work, the miniemulsion polymerization of methyl methacrylate was optimized to produce PMMA particles for encapsulation of natural extracts with therapeutic potential in diseases such as leukemia and leishmaniasis. PMMA nanoparticles were used in cultures of immortalized cells of leukemic origin (K562[13]) to investigate possible toxicity of these particles.

Materials and Methods

Miniemulsion Stability

Miniemulsion stability was analyzed at room temperature by light profiling using Turbiscan. This technique allows for the scanning of transmitted and scattered light of emulsions or suspensions in various positions along the sample. Analysis results are the light transmission and backscattering profiles from the sample in function of the height of the flask, from the bottom to the top of the tube.

Nanoparticle Preparation

Poly(methyl-methacrylate) was produced using miniemulsion polymerization in order to obtain nanometric polymer particles.[20–24] The organic phase consisting of 29.1 g of methyl methacrylate and 0.9 g of hexadecane was mixed in a becker on a stirring plate for 5 minutes at 150 rpm. This solution was added to another becker containing the aqueous phase consisting of 120 g of deionized water, 0.5 g of potassium persulfate, 1.5 g of sodium dodecyl sulfate (surfactant) and 0.2 g of sodium bicarbonate. Then, it was stirred for 3 minutes at 150 rpm. This dispersion was sonicated by an ultrasound probe (Sonics model VCX750) for 4 minutes at an amplitude of 40%. The miniemulsion obtained was transferred to the polymerization reactor at 80 °C where the polymerization took place for 3 hours. Approximately 100% of conversion, measured by gravimetry, was obtained in this condition. The polymer latex was freeze dried or lyophilized using a Beta 1-6 Christ Freeze Dryer. Reversible particle agglomeration was observed after lyophilization.

Polymer Characterization

Polymer chemical characterization was performed by Fourier transform infrared spectroscopy (FT-IR) using KBr pellets. PMMA thermal behavior was measured by differential scanning calorimetry (DSC) in a PerkinElmer Diamond DSC equipment, using nitrogen as gas carrier at a flow rate of 80 mL.min^{-1}. Approximately, 3 mg of polymer were melted, cooled at 10 °C min^{-1} and then the thermogram was recorded from 25 °C to 200 °C at 10 °C.min^{-1}.

Polymer molecular weight distribution analysis was measured in a Viscotek Model VE2001 equipment using a refractive index detector (VE 3580) and a set of Phenomenex columns (500 Å; 10,000 Å; 100,000 Å and 1,000,000 Å). Tetrahydrofuran (THF) was used as mobile phase at a flow rate of 1 mL min^{-1} at 40 °C. Samples were prepared by dissolving approximately 10 mg of polymer in 10 mL of THF and an injection volume of 100 μL was used.

Nanoparticle morphology was evaluated by scanning electron microscopy (SEM) using a Quanta 200 FEI microscope. The polymer sample in a spatula tip was poured on a double-sided adhesive tape on a sample support. The support was taken to gold metallization under high vacuum and taken to the microscope. Particle size was measured by dynamic light scattering using Zeta-sizer Nano ZS.

Activity in Cell Culture

Polymer nanoparticles (freeze dried) were suspended in a culture medium and then filtered using a sterile filter of 45 mm diameter and 0.22 μm pore size. The study of antitumor activity was performed using human leukemic cell line K562[13] at a concentration of 5×10^5 cells mL^{-1}. The medium used for the cell growth was D-MEM and RPMI 1640 with 10% fetal bovine serum (FBS), antibiotics (penicillin and streptomycin), antimycotic (amphotericin B) and polymer nanoparticles (750 μg mL^{-1} to 1500 μg mL^{-1}). The cells were incubated for 24 hours in a CO_2 incubator at 37 °C. Cytotoxicity activity was evaluated using trypan blue test and the MTT assay (colorimetric assays for measuring cell viability and proliferation) reagents, as described by Campos *et al.*[2]

Results and Discussion

Few studies in the literature analyze the stability of miniemulsions used for polymerization. Figure 1 shows the light transmission and scattering profile from the miniemulsion used in the polymerization at different times. Figure 1-A shows that the emulsion is opaque since there is no light transmission. The moderate increase of the backscattering profile (Figure 1-B) with time indicates that the miniemulsion may be regarded stable within one hour, with a small increase in the average droplet size probably due to diffusional degradation. Besides, after one hour, the sample showed a small peak at the top of the bottle (Figure 1-B), indicating a higher concentration of droplets at the top of the flask due to creaming. These results suggest that the miniemulsion is moderately stable at room temperature, therefore the polymerization reaction should be carried out rapidly to allow the production of particles in the nanometer scale. It is important to notice that polymerization reactions are usually carried out at higher temperatures, and even lower emulsion stability should be observed in this condition.

Miniemulsions used in polymerization processes are not necessarily stable and are subject to various phenomena such as flocculation, Otswald ripening (diffusional degradation) and coalescence. According with Schork *et al.*,[12] emulsions can be stabilized against diffusional degradation by incorporating a polymeric costabilizer, and the presence of large numbers of small droplets, as in miniemulsions, shifts the nucleation mechanism from micellar or homogeneous nucleation to droplet nucleation contributing to emulsion stability while allowing for easier encapsulation of materials.

Monomer droplets showed a zeta potential in the range of -42.50 ± 2.21 mV that reinforces the hypothesis the miniemulsion has some degree stability with little tendency to flocculation or coalescence. According to Greenwood,[25] the value of the zeta potential can be related to the colloidal stability as it indicates the degree of electrostatic repulsion between dispersed particles. If particle absolute zeta potential value is larger than 30 mV, it indicates a stable formulation[26] with good resistance against particle aggregation. When the value of the zeta potential is lower, van der Waals attraction overcomes electrostatic repulsion and dispersed particles will aggregate, coagulate or flocculate. Therefore, the miniemulsion produced is relatively stable against flocculation and can be used in the polymerization process to produce poly(methyl methacrylate) nanoparticles.

Polymer particles in the latex showed an average diameter of 90.4 ± 4.1 nm with polydispersity index of 0.20 ± 0.08. A

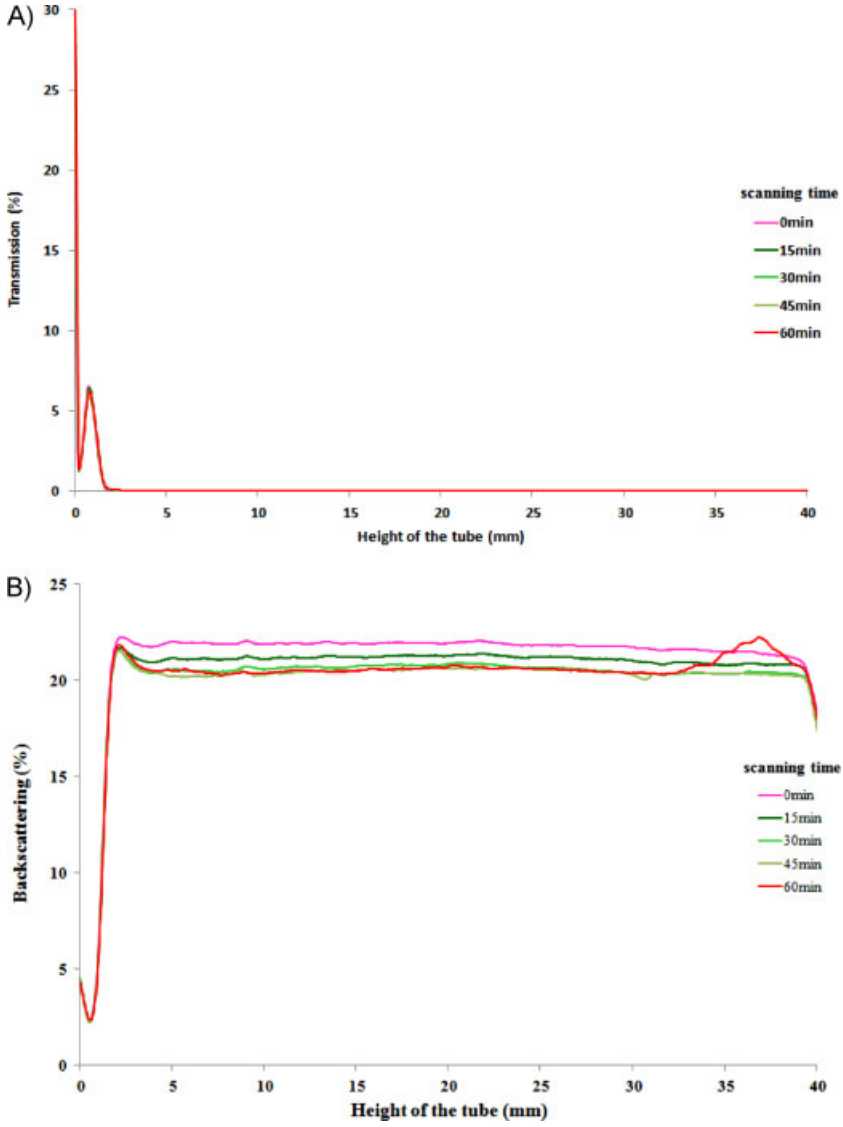

Figure 1.
Miniemulsion light transmission (A) and backscattering (B) profiles in function of time.

similar particle size was obtained for the dried polymer after sonication. The dynamic light scattering particle size analysis was confirmed by the SEM (Figure 2) that gave an estimated average particle size of 100 nm with spherical morphology, as expected.

Surfactant type and concentration can directly influence the particle size. Literature data showed that SDS can generate negative surface charges and, at a concentration of $10\,g\,L^{-1}$, PMMA nanoparticles can be produced, with an average diameter of 55 nm,[12] using poly(methyl methacrylate) as costabilizer.[12] In this work, the costabilizer was the hexadecane and a higher particle size was obtained.

FT-IR spectrum (Figure 3) showed signals in 2990 and $2950\,cm^{-1}$ that can be assigned to axial deformation of CH bonds

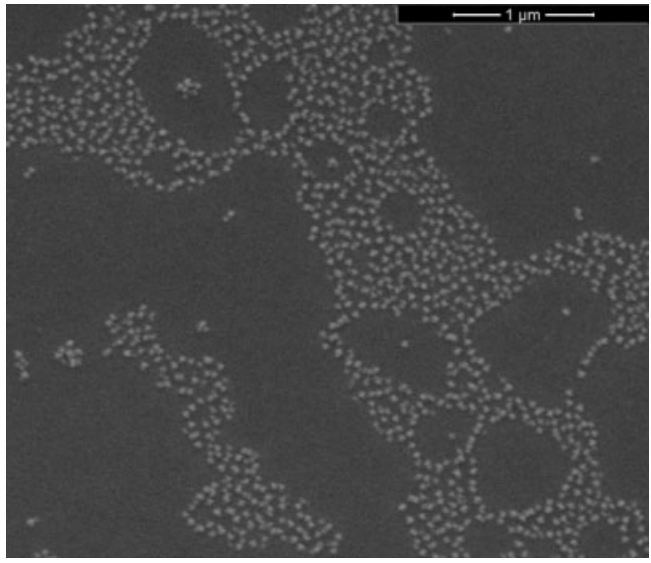

Figure 2.
Scanning electron micrograph of PMMA nanoparticles. (65,000 x).

of aliphatic carbons while the signal around 1430 cm^{-1} can be assigned to the angular deformation of CH bond. The sharp and intense signal at 1724 cm^{-1} can be assigned to the axial deformation of the carbonyl group (C=O). The presence of this sharp signal suggests a homogeneous polymer sample. The region comprised in the range

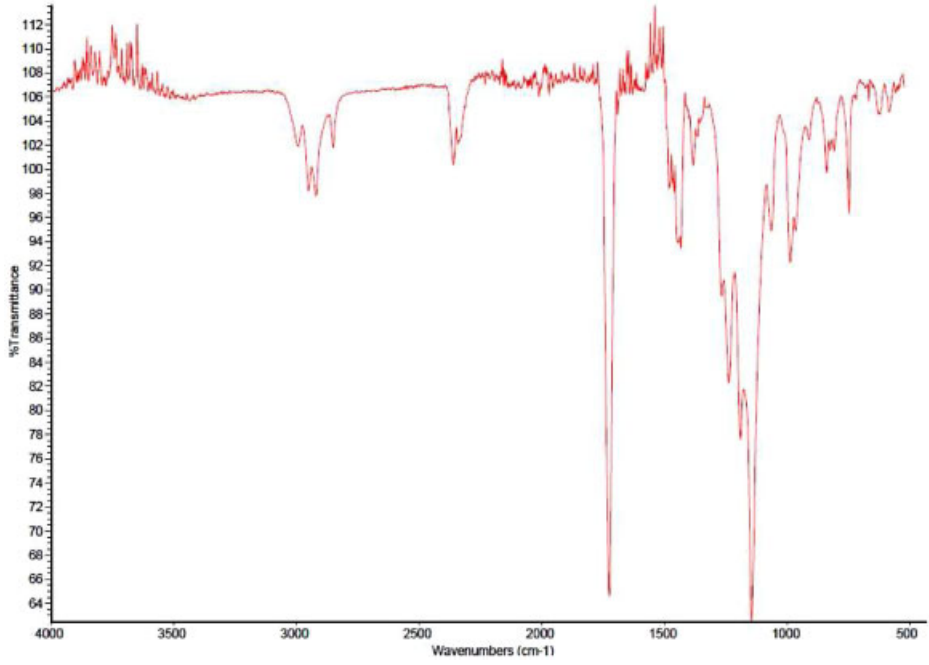

Figure 3.
FT-IR spectrum of PMMA (Poly (methyl methacrylate).

Table 1.
K562 cells viability in the presence of PMMA nanoparticles.

Concentration ($\mu g\,mL^{-1}$)	Cell viability (%)		
	1 h	24 h	
	Tripan blue[a)]	Tripan blue[b)]	MTT[c)]
Control[d)]	89	89	100
DMSO[e)]	94	91	92
750	82	94	100
1500	82	90	100

[a)]Cell viability (Trypan Blue) after 1 hour of treatment.[b)]Cell viability (Trypan Blue) after 24 hour of treatment. [c)]Cell death (MTT) after 24 hours of treatment.[d)]Control: cells that were incubated in absence of DMSO or nanoparticles.[e)]DMSO: cells that were incubated in a medium containing 0.01%w/w of dimethyl sulfoxide.

of 1250-1000 cm^{-1} showed signals related to the angular deformation of C=O in ester bonds. These data are consistent with the literature for PMMA.[6]

Nanoparticles were used for toxicity testing in human leukemic cells (strain K562). The polymer nanoparticle were suspended in a culture medium and incubated with K562 cells at different concentrations ($750\,\mu g\,mL^{-1}$ to $1500\,\mu g\,mL^{-1}$). After incubation cytotoxity was measured at 1 and 24 hours by two different methods (trypan blue and MTT[2]). The results indicated that the PMMA nanoparticles presented no cytotoxicity to K562 cells, with cell viability values near those obtained by the control groups (Table 1).

PMMA average glass transition temperature (Tg) was $97.0 \pm 4.4\,^{\circ}C$, within the range reported in the literature.[9,10] The observed average values of number and molecular weight were 58.12 ± 4.41 kDa and 419.09 ± 0.67 kDa, respectively.

Conclusion

The preparation of PMMA nanoparticles by miniemulsion polymerization produced particles of about 90 nm and the original miniemulsion showed moderate stability. The obtained nanoparticles did not present any observable toxicity when tested against human leukemic cell line K562. These results are relevant for the development of antitumor agents encapsulated in PMMA nanoparticles.

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