Biodegradable Micro and Nanoparticles for Controlled Drug Release

C. Mattu[*], S. Sartori[*], E. Ranzato[**], M. Boffito[*], R. Li[*] and G. Ciardelli[*]

[*]Politecnico di Torino, Dipartimento di Meccanica
C.so Duca degli Abruzzi, 29 – 10129 Torino, Italy – clara.mattu@polito.it

[**] Department of Environment and Life Sciences, DiSAV,
University of Piemonte Orientale "Amedeo Avogadro",
viale T. Michel 11, 15121 Alessandria, Italy

ABSTRACT

In this paper, we propose the encapsulation of the anticancer drug Paclitaxel into biodegrdable polymeric carriers to improve the release properties of the active principle.
Commercial polyesthers, such as Poly-ε-Caprolactone (PCL) or Poly Lactic Acid (PLA) and a series of proprietary polyester-urethanes (PU) were selected to prepare micro or nanoparticles for controlled drug release.
The Solvent Displacement Method or the Oil in Water single Emulsion Solvent Extraction Evaporation Method were optimized to prepare colloidal carriers with tunable release profiles and encapsulation properties.

Keywords: microparticles, nanoparticles, paclitaxel, polyurethanes, polyesters

1 INTRODUCTION

Biodegradable polymeric micro and nanoparticles have shown to be promising forms for the delivery of a wide array of drug formulations, such as hydrophobic or hydrophilic active principles, proteins or other bio molecules [1]. Their ability to sustain a controlled release for a prolonged period of time at a specific site of action makes them suitable candidates for the selective delivery of chemotherapy, overcoming common problems associated with high systemic toxicity and poor bioavailability of traditional therapies.

In addition to the above mentioned properties, nanoparticles offer some advantages over microparticles such as higher carrier capacity, superior stability and the ability to passively target tumour cells through the Enhanced Permeation and Retention (EPR) Effect [2]. Tumours are composed of fastly-growing cells which need an extensive supply of oxygen and other nutrients. Their vessels are therefore highly fenestrated (200-300nm) and allow small-size particles to accumulate inside cancer cells. Moreover tumours lack of an efficient lymphatic system and are not able to eliminate these particles once they entered the cells.

The aim of this study was to prepare micro and nanospheres starting from commercial polyesters, such as Poly-ε-Caprolactone (PCL) and Poly Lactic Acid (PLA) or proprietary Polyester-urethanes (PU), for the controlled and targeted delivery of the anticancer drug Paclitaxel (PX) [3].
PUs are an interesting group of polymers with versatile characteristics, such as tuneable mechanical properties and good biocompatibility, depending on the molecular composition. Changes in the molecular weight or chemical structure of their soft and hard segments allow for optimization of mechanical and chemical properties of these materials. Polyurethanes have been successfully employed as biomaterials for several decades; nevertheless little or none is reported in the literature concerning their use as particles for controlled drug release.

2 MATERIALS & METHODS

2.1 Polyurethane synthesis

Polyurethanes used in this study were prepared starting from amphiphilic tri-block poly(ε-caprolactone)-poly(ethylenglycol)-poly(ε-caprolactone) copolymer (PCL-PEG-PCL) and PCL diol, following a two step synthesis procedure [4,5,6].
Poly(ε-caprolactone) diol (PCL, M_n=1250 or M_n=2000, Aldrich) and low molecular weight tri-block PCL-PEG-PCL copolymers (CE-650 and CE-635) were used as soft segments. 1,4-cyclohexane dimethanol CDM (Aldrich, mixture *cis* and *trans*) was used as chain extender. Two different diisocyanates were selected and distilled before use: Methyl-2,6-diisocyanatehexane (LDI) and 1,4 butanediisocyanate (BDI) (see table 1).

2.2 Microparticles preparation

PX-loaded PU microspheres (5%w/w) were prepared by the Oil in Water Single Emulsion Solvent Extraction Evaporation Technique, using Polyvinylalcohol (PVA) and TWEEN 80 (Sigma Aldrich) as emulsifiers [7]. Briefly, 300 mg of polymer and 15 mg of PX were dissolved in 5 ml dichloromethane (DCM) and added to 100 ml water solution of PVA. This oil-in-water solution was stirred at

800 rpm for 2 h, to allow solvent evaporation. The final product was collected by selective centrifugation, washed to remove the excess of emulsifier and finally freeze-dried.

Polymer	Composition
CLCE-650	CDM, LDI, PCL-PEG-PCL (CE-650 PEG M_w= 600,)
CLCE-635	CDM, LDI, PCL-PEG-PCL (CE-635)
CE-35034	PCL-PEG-PCL (PEG M_w= 35000)
CLC-1250	CDM, LDI, PCL (M_n= 1250)
CLC-2000	CDM, LDI, PCL (M_n= 2000)
ELC-2000	Lesine ethyl ester, LDI, PCL (M_n= 2000)
CBC-2000	CDM, BDI, PCL (M_n= 2000)

Table 1: List of polymers with their composition, used for microsphere preparation.

2.3 Nanoparticles preparation

In an attempt to improve the release properties of the micro-carriers and take advantage of the superior stability, carrier capacity and passive targeting ability of smaller-sized particles, PX loaded nanocarriers (3% w/w) were prepared by the solvent displacement technique [8] using PCL (Mw 60000 polysciences), PLA (Mw 40000 Polysciences) or a newly synthesised low molecular weight Polyesterurethane (CBC 2000, Mn 10000 - our GPC measurement) as matrix-forming materials and TWEEN 80 as emulsifier. For nanoparticles preparation, the polymer and the drug were dissolved in 10 ml acetone, at an optimized polymer concentration of 0.6% w/v. This solution was added drop wise into 20 ml of water containing the emulsifier (0.3% w/v). Particles formed instantaneously for the fast diffusion of the solvent from the core of the droplets towards the water phase. After dialysis (48h) particles were collected by centrifugation and freeze-dried in the presence of Trehalose as cryo-preserving agent.

2.4 Cellular tests

A431 cells (human epithelial carcinoma cell line) were cultured in DMEM supplemented with 10% foetal bovine serum (Sigma-Aldrich) and 1% antibiotic mixture (Sigma-Aldrich). To assess toxicity of nanoparticles the calcein-am assay was used. This viability test uses the lipophilic, nonfluorescent calcein-acetoxymethylester (Cal-AM), which penetrates cell membranes and is then cleaved by intracellular esterases, yielding the hydrophilic fluorescent dye. Cells were washed with PBS and then incubated for 30 min at 37°C with a solution of 2.5 µM Calcein-AM in PBS. Plates were read with a fluorescence reader (Infinite Pro200, Tecan, Wien, Austria) by using 485-nm exc and 535-nm em filters.

To assess the ability of nanoparticles to penetrate cells membrane, Rhodamine (Aldrich)-loaded nanoparticles were prepared, by adding the dye (1% w/w) to the polymer-acetone solution. After 24 h of exposure, cells were fixed with 3.7% paraformaldehyde, permeabilized with 0.1% Triton X-100, and actin was stained with FITC-phalloidin. Cells were observed with a Zeiss LSM 510 confocal system interfaced with a Zeiss Axiovert 100 M microscope (Zeiss, Oberkochen, Germany).

3 RESULTS & DISCUSSION

Biodegradable micro and nanoparticles were successfully prepared for the controlled release of Paclitaxel. All particles were characterized in terms of size distribution, morphology (SEM) and release kinetics.

3.1 Pu Microparticles

SEM (Leo Instruments) micrographs (figure 1) showed that regardless of the parameters adopted, particles in all cases had a regular morphology with a dimple, non porous surface. No coalescence was observed in any case.

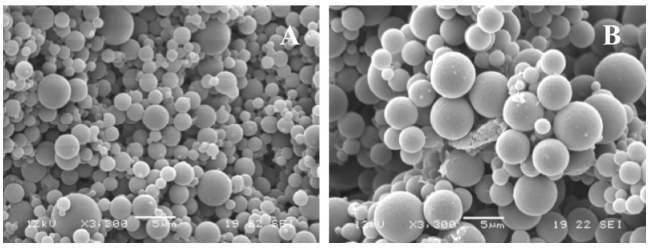

Figure 1: PX-loaded microspheres: A) CLCE-635 and B) CLC-1250.

Depending on the material used and the parameters adopted, microparticles with a diameter from 1 to 100µm were obtained.

Size, Resuspension index (RI) and Encapsulation efficacy (EE) of the particles appeared strongly dependent on the polymer composition: the presence of the hydrophilic PEG segment is correlated to a reduction in particle size and EE. RI varied from 10% to 100% with the highest values obtained for PEG containing polymers. EE values ranged from 25% to 97% depending on the hydrophobicity of the matrix forming material.

PX release kinetics were evaluated in PBS for 34 days and showed a nearly linear, almost zero-order release profile (figure 2) with a low initial burst effect associated with reduced systhemic toxicity of the drug (1% for CLC-2000; 2.9% for CE-35034; 6.7% for ELC-2000; 7.7% for CLCE-635 and 8.4% for CLCE-650).

Release kinetics can be related to the high molecular weight, the large particle dimensions and the absence of PEG segments in the polymer chain [9].

Release was faster for particles prepared with PEG-containing polymers probably due to the swelling of the matrix, which favours drug diffusion.

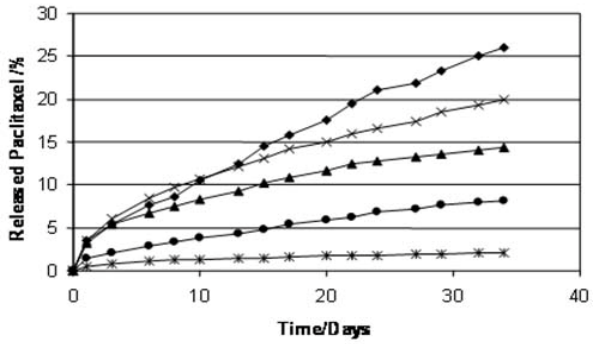

Figure 2: In vitro drug release kinetics from 5% Paclitaxel loaded microspheres (♦ CLCE-635, × CLCE-650, ▲ ELC-2000, ● CE-35034, * CLC-2000)

3.2 Biodegradable Nanospheres

Nanoparticles were successfully obtained by the Solvent Displacement Method, as confirmed by SEM micrographs (reported in figure 3), with both commercial polyesthers and the low molecular weight Poly-Esther Urethane CBC 2000. All particles exhibit nanosized dimensions and a distinct and well-defined spherical shape with little or none coalescence**.**

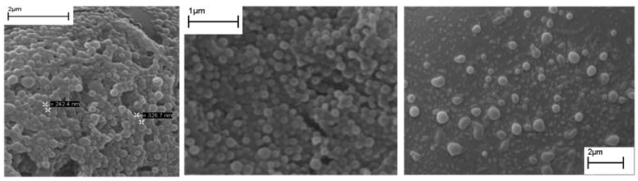

Figure 3: PX-loaded (3%) nanospheres, prepared with different materials: A) PCL, B) PLA, C) CBC2000.

Results of particles size analysis, assessed by Dynamic light Scattering (Malvern Zetasizer Nano S90), are summarized in Table 2. Regardless of the polymer used, particles with small size (lower than 250 nm) and low Polydispersity Index (PDI) were obtained.
Particles obtained with the newly synthesised low molecular weight polyurethane CBC-2000 show a regular spherical morphology and a slight increase in size and PDI, when compared to particles obtained with commercial polyesters.

MATERIAL	Mean Size [nm]	PDI
PCL	**211 ± 36**	**0,11 ± 0,04**
PLA	**213 ± 27**	**0,13 ± 0,02**
CBC-2000	**225 ± 13**	**0,18 ± 0,04**

Table 2: Particles size Analysis resuls (mean ± SD) n = 3.

No signs of toxicity after proper dilution of all samples, were detected with Calcein-am assay, which showed a similar pattern for nanoparticles prepared with different materials. The confocal analysis revealed that nanoparticles were internalized by cells and localized almost totally in the cytoplasm, as revealed by fluorescence of Rhodamine-conjugated nanoparticles (Figure 4).

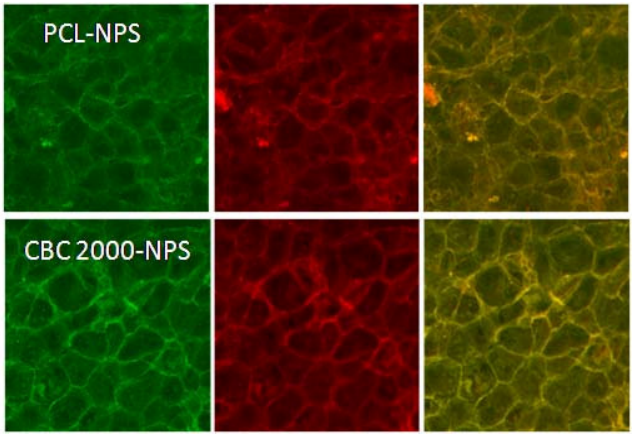

Figure 4: A431 cells at confocal microscopy (20X). Fitc-phalloidin for actin staining (Left), Rhodamine-loaded nanoparticles (middle) and dual color images showing the cytoplasmic localization of particles (Left)

Pacltaxel-loaded nanoparticles were sucessufully prepared and characterized in terms of Drug Loading Efficiency (DL%) and release properties. As reported in table 3, particles prepared with the newly synthesised polyuretane CBC 2000 showed a higher drug loading efficiency (89%) if compared to particles prepared with commercial polymers (18% and 24% for PCL and PLA respectively).

MATERIAL	DL %	
	Mean	Std Dev (n = 3)
PCL	**18%**	**8%**
PLA	**24%**	**7%**
CBC-2000	**89%**	**2%**

Table 3: Drug loading result for nanoparticles prepared with different polymers.

Release profiles from nanoparticles, over a period of 48 hours, are presented in figure 5 and show that CBC 2000 nanoparticles were able to sustain drug release for a prolonged period of time, while release from PCL or PLA nanoparticles was completed within the first 48 hours. Particles prepared with the two commercial polyester had a similar profile with an initial burst effect in the first hour, followed by a liner tract for the first two days, when all the encapsulated drug was released. CBC 2000 nanoparticles, showed the highest drug loading capacity and the lowest burst effect, indicating that this polymer allowed us to obtain nanoparticles with optimal release properties, such as high drug loading capacity, low initial burst and prolonged release over extended periods of time.

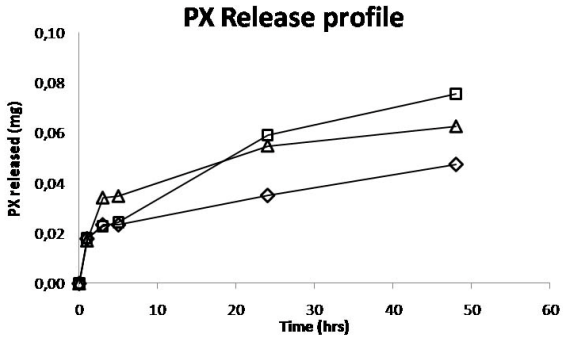

Figure 5: Release profile of Paclitaxel from: □ CBC 2000 nps, Δ PLA nps and ◊ PCL nps

4 CONCLUSIONS

In this work, micro or nanoparticles prepared with degaradable polyesters or novel synthesised biomaterials were proposed for the controlled and targeted release of Paclitaxel.

Polyurethanes have been successfully employed as biomaterials for several decades in a variety of applications, because of their tuneable mechanical properties and good biocompatibility; nevertheless their use as particles for controlled drug release is not reported in literature.

The Oil in Water Single Emulsion Solvent Evaporation Technique allowed us to obtain microparticles of regular morphology. These microspheres showed high encapsulation efficiency and a linear release profile over 34 days, with a low burst effect.

In an attempt to improve the targeting properties of the carriers by taking advantage of the EPR effect, nanoparticles were prepared and characterized. The solvent Displacement method was adopted for this purpose, because it is known to produce small and uniform particles without employing toxic solvents. Spherical nanoparticles with uniform size distribution were successfully obtained with commercial polyesters and with a low molecular weight innovative poyesterurethane. The size, assessed by Dinamic Light Scattering, makes the particles optimal candidates for passive targeting of tumours through the EPR effect, as shown by cellular tests. Nanoparticles are easily internalized by cells after 24h of incubation and accumulate in the cell's cytoplasm.

Release profiles of PX were similar for nanoparticles prepared with commercial polyesters with an initial burst release followed by a linear tract. Almost all the encapsulated drug was released after 48 hours. CBC 2000 nanoparticles exhibited optimal release properties, with the highest drug loading capacity, the lowest burst effect and a prolonged release over time.

This study highlighted the possibility to successfully employ novel biomaterials as particles for controlled drug release and to modulate their release profiles and encapsulation properties by selecting the suitable soft segments of the polyurethanes.

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