Preparation and evaluation of poly (caprolactone fumarate) nanoparticles containing doxorubicin HCl

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ABSTRACT

Background and the purpose of the study: Biodegradable Poly(caprolactone fumarate) (PCLF) has been used as bioresorbable sutures. In this study, doxorubicin HCl (Dox) loaded PCLF nanoparticles were prepared and characterized.

Material and methods: PCLFs were synthesized by polycondensation of PCL diols (Mws of 530, 1250 and 2000) with fumaryl chloride. The degradation of PCLF in NaOH, water and phosphate buffer saline (PBS), was determined in terms of changes in Mw. Nanoparticles (NPs) were prepared by two methods. In microemulsion polymerization method, dichloromethane containing PCLF and photoinitiator were combined with the water containing surfactants and then the mixture was placed under light for crosslinking. In nanoprecipitation method, the organic solvent containing PCLF was poured into the stirring water. The effect of several variables including concentration of PCLF, polyvinyl alcohol (PVA), Dox and Trypan blue (Trb) and the Mw of PCLF and PVA on NP size and loading were evaluated.

Results: PCLF 530, 1250 and 2000 in PBS or water were not degraded over 28 days. Nanoprecipitation method gave spherical (revealed by SEM images) stable NPs of about 225 with narrow size distribution and a zeta potential of -43 mV. The size of NP increased significantly by increase in Mw or concentration of PCLF. Although PVA was not necessary for formation of NPs, but it decreased with NP size. Dox loading and EE were 2.5-6.8% and 15-20%, respectively. Increasing the drug concentration increased the drug loading (DL) and NP size. The entrapment efficiency (EE) for Trb ranged from 1% for PCLF530 to 6% for PCLF2000. An increase in PCLF concentration resulted in an increase in EE. Dox and Trb release showed a burst followed by 80% and 78% release during 3 and 4 days respectively. Conclusion: PCLF possessed suitable characteristics for preparation of nanoparticulate drug delivery system such as desired NP size, stability and degradation time. Although PCLF530 NPs were the smallest, but their DL were lower than PCLF1250 and 2000 NPs.

Keywords: PCLF nanoparticles, Copolymer molecular weight, Nanoprecipitation method.

INTRODUCTION

Polymers and polymeric NPs are promising vehicles for drug delivery. Their important properties are easy manipulation to prepare carriers with the objective of delivering drugs to the specific target (1). Such an advantage improves the drug safety (1) which is particularly important for cancer therapy by reducing wide side effects of the anticancer drugs. One way to achieve this goal is the passive targeting of the active molecules to the tumor site, which is mainly based on the enhanced permeability and retention (EPR) effect. It means that the polymeric NPs in comparison with the free drugs are accumulated in the tumor tissues more than normal tissues due to high permeability and low drug elimination related to tumor sites. Moreover, NPs can prolong blood circulation time and control the release rates of drugs in tissues (2,3). Poly (caprolactone) (PCL) is a hydrophobic biocompatible and biodegradable polyester which has been used for bioresorbable sutures and scaffolds (4) and for micro and nanoparticulate drug delivery systems (3,5). PCL biodegradability can
be improved by proper copolymerization strategies (6,7). Several copolymers have been synthesized from PCL and a hydrophilic segment such as poly(ethylene glycol) (PEG) (8,9). Hydrophilic fumaric acid has been utilized by Shin et al to synthesize oligo (PEG fumarate) (OPF) for hydrogel implants by crosslinking the double bond of fumarate. Grijpma et al have reported synthesis of star-shaped oligomers functionalized with fumaric acid monoethyl ester (FAME) and preparation of networks by its photocrosslinking. Wang et al copolymerized fumarate with PCL as poly(caprolactone fumarate) (PCLF, Fig 1) copolymers and used it to prepare implants for tissue engineering.

Since particulate systems of PCLF have not been reported, in this study, synthesis of PCLF according to Wang et al (12,13) and its uses for preparation of NPs suitable for drug delivery was investigated. In addition, since macrophages in reticuloendothelial system (RES) tend to phagocyte more hydrophobic molecules, it seemed that hydrophobic PCLF NPs can enhance lymphatic uptake and to be useful for lymphoma therapy by passive targeting (14,15). The hydrophobicity of PCLF can be easily tunable via synthetic process by using precursors of different Mws and probably PCLF may encapsulate more hydrophobic drugs in comparison with simple PCL.

Self-crosslinkable PCLF may adjust drug loading and release of the NPs (16). Myocet and Doxil are liposomal dosage forms of doxorubicin HCl and fumaryl chloride, PCLF530, PCLF1250 and PCLF2000 (named after their precursors) were synthesized through a polycondensation reaction according to the reported method (12,13) using PCL diols with Mw of 530, 1250 and 2000 g mol⁻¹, fumaryl chloride and K₂CO₃ (proton scavenger) and identified by ¹H-NMR and FTIR. PCLF Mw and melting point (T_m) were determined by Gel Permeation Chromatography (GPC) and Differential Scanning Calorimetry (DSC) spectra, respectively according to the previously reported method (18). The ¹H-NMR spectra of copolymers were recorded on a Bruker 500 MHz or Varian Unity Plus 400 (300 MHz) or a Bruker 80 MHz spectrometers using DMSO-d₆ as solvent. IR spectra were obtained using a Nicolet FT-IR Magna 550 spectrophotometer. The GPC device was from Knauer Co., with refractive index detector 2300, and the used column was Knauer AXX10 column, 1510-5330 PKL gel mixed E 3µm 250x4 6mm. A calibration curve constructed by a series of polystyrene standard using THF as eluant, was used to estimate the Mw of copolymers. DSC scans were obtained by a Mettler DSC 823 (Mettler Toledo GmbH, Switzerland) with Mettler Star® Software, Version 9.x. PCLF. For in vitro degradation test, 30 mg of the sieved powder of PCLF2000 was immersed in 30 ml of different media in sealed glass tubes at 37°C. PCLF and medium samples were taken at certain time intervals and the PCLF Mw and fumaric acid concentration were measured using GPC method (19) and UV spectrophotometer at 207 nm, respectively.

**Preparation and characterization of PCLF NPs**

**Microemulsion polymerization method**

Two ml of DCM and/or MMA as organic solvent containing PCLF (0.5-1 w/v%) and CQ (crosslinking photo-initiator) (0.005 w/v%) was poured into 50 ml of solution of dermaphyl as surfactant in water (4-24% v/v) with or without polysorbates 80 and 20 (5-15% v/v) under stirring, and placed under visible light for one hour.

**Nanoprecipitation method**

Two milliliters of a solution of PCLF in acetone (0.1-6% w/v) was poured into 4 ml of water solution of PVA (0.01-0.6% w/v) and drug (0.02-0.4% w/v) under stirring. The resulting NPs suspension was passed through a 0.45 µm sterile filter and NPs were isolated by 0.1 µm membrane filter (PC type, Whatman). NPs were washed by DW and then redispersed in DW to check their stability. NPs suspension was lyophilized (Lyotrap plus freeze dryer, LTE scientific co., UK) to obtain a powder.

**Chemical structure of PCLF.**

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[Image of chemical structure of PCLF]
Table 1. Physical characteristics of PCL diols and PCLFs.

| Macromer polymer | \( \bar{M}_n \) (g/mol) | \( M_w \) (g/mol) | PDI | \( T_m \) (°C) |
|------------------|---------------------------|-------------------|-----|--------------|
| PCL diol 530     | 792                       | 1218              | 1.5 | ___          |
| PCL diol 1250    | 2844                      | 4010              | 1.2 | 60           |
| PCL diol 2000    | 3092                      | 6339              | 2.0 | 63           |
| PCLF530          | 4019                      | 6026              | 1.4 | ___          |
| PCLF1250         | 6960                      | 9287              | 1.3 | 57           |
| PCLF2000         | 8455                      | 11623             | 1.3 | 59           |

\( \bar{M}_n \), number average molecular weight; \( M_w \), weight average molecular weight; PDI, polydispersity index; \( T_m \), melting point.

The effects of several variables including the concentration of the components, \( M_w \) of PCL diol and PVA (22000 and 72000 g/mol), type of the solvent (S) or non-solvent (NS) and the S/NS ratio (0.02-1) as well as some process parameters on NP size, polydispersity index (PDI), zeta potential (laser light scattering, PCS), Malvern Zetasizer, Nano-ZS series, Malvern Co., UK) and drug loading (DL) were investigated. Each variable was changed while others were constant.

The NPs size, shape and surface morphology were checked by Scanning Electron Microscopy (SEM, Philips XL 30 scanning microscope, Philips Co., the Netherlands). SEM samples were prepared by dropping 200 μl of each NP suspension onto a standard glass surface and removal of water under reduced pressure.

The DL and the Entrapment Efficiency (EE) were calculated as follows:

\[
\text{EE} \, (\%) = \frac{\text{Amount of drug in NPs}}{\text{Total amount of drug}} \times 100
\]

\[
\text{DL} \, (\%) = \frac{\text{Amount of drug in NPs}}{\text{Amount of drug-loaded NPs}} \times 100
\]

The amount of drug in NPs was determined by subtraction of the unloaded drug from the total drug that initially was added to the solution. The unloaded drug concentration, was measured by UV/VS (Jarso V-530, Jasco co., Japan) absorbance at 586 and 233 nm for Trb and Dox, respectively after separation of NPs by 0.1 μ filter.

NPs suspended in DW, were tested for size at room temperature (RT) at certain time intervals without considering the precipitates (if any). For the drug release experiments, 12 mg of lyophilized NPs (formulations 15 or 21, Table 4) was suspended in a 10 ml of DW loaded in a dialysis bag and placed in 30 ml of PBS and then positioned on a shaker incubator (Heidolph incubator 1000, Heidolph co., Germany) at 37°C and 70 rpm. At selected time intervals, 5 ml samples were taken and replaced with 5 ml of fresh PBS.

**Statistical analysis**

The data were presented as mean ± SD. One-way analysis of variance (ANOVA) was used for comparing the mean differences. SPSS for Windows (release 11.5.0) was employed for statistical analyses and P value <0.05 was considered significant. All experiments were performed in triplicates, and the averages values were taken into considerations.

**RESULTS AND DISCUSSION**

**Synthesis and Characterization of PCLFs**

The \(^1\)H-NMR, FTIR, GPC and DSC spectra data of PCLFs were in accordance with the reported data by Wang et al (12). The \( M_w \), PDI and \( T_m \) of the PCL diols and PCLFs are given in table 1.
polymers) enough to show a sustained release of drug (21,22) which is related to its synthetic origin (23). Moreover PCLF exhibits a high level of hydrophobicity (22) which may inhibit the access of water to the polymer during the degradation (24, 25).

**Microemulsion polymerization method**

Experiments revealed that by this method, formation of NPs was highly dependent on the exact concentrations of the used materials. It has also been reported that the size of NPs produced is dramatically influenced by the composition of the polymerizing mixture especially the type and the concentration of the emulsifier (17). Most of the produced NPs were unstable in their aqueous suspension, aggregate and precipitate after a few hours to one day. Among many prepared formulations by different concentrations of copolymer or surfactant, different types of surfactants and different S/NS ratios, few of them remained stable as suspension during a few days indicating slower aggregation. Such formulation of NPs were made by addition of 0.2 ml MMA containing CQ and PCLF2000 (1% w/v) into 50 ml of dermaphyl (10% in DW), representing NP size of 191.7±40.3 nm, PDI of 0.27 and zeta potential of -23.4±10.7 mV. Also another formulation

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**Figure 2.** Change of PCLF2000 Mw, in different mediums during 28 days (n=3).

**Figure 3.** Cumulative percentage of the released fumaric acid from PCLF2000 in different mediums during 28 days (n=3).
composed of 2 ml of MMA containing CQ PCLF2000 (1 w/v%), and 50 ml DW containing 10% dermaphyl and 10% polysorbate20, had NP size of 403.1±67.8 nm and, PDI of 1.0±0.3. NPs prepared by this method showed decrease in diameter by increase in concentration of dermaphyl (1-10% as surfactant). This finding confirms the results of another report for span 65 as the surfactant to prepare PEG-PPG-PEG NPs by inverse emulsion polymerization method (17). By increasing the copolymer concentration above 1%, the particles were large and resulting suspension was unstable and precipitated.

**Nanoprecipitation method**

In this method, NPs were produced immediately and remained as stable suspension as it was confirmed by nanosizer Malvern Co. (UK), and on the contrary of the microemulsion polymerization method, produced NPs over a wider range of concentrations of the compositions and different ratios of solvents. Such flexibility made the method easier to design for different purposes. Moreover, this method resulted in more re-dispersable and more stable NPs than other method. In addition, the number of materials which were used in this method and their toxicities were lower than the microemulsion polymerization method which was consistent with results of another report (26). Additionally the use of PVA was not necessary for NPs formation in this method (formulation 6, Table 2), which is important for intravascular delivery. The lower PDI and SD of NP size obtained by repeated formulations of this method (0.1 and 7.8 respectively for formulation 8), than the microemulsion polymerization method (0.27 and 40 respectively), demonstrated a higher reproducibility of the nanoprecipitation method and a narrow distribution pattern of the NPs (Fig 4), confirming the report of Bilati et al (26). Therefore, this method was chosen to investigate preparation and characterization of PCLF NPs. Table 2 shows when the solvent and non-solvent are acetone and DW, respectively, the NPs size was 117-216 nm (formulation 6-13). The negative zeta potential of the NPs was probably due to the residual carboxylic acid groups of the fumaric acid terminated polymers, as for the PLGA. Control formulation (formulation 8 without PCLF) produced NPs with sizes of 318.1±11.0 nm, PDI of 0.5±0.0 and zeta potentials of -1±0.4 mV. Therefore such a formulation may be just clusters of PVA molecules. A solution of PCLF in acetone and a water solution of PVA, both exhibited no significant NPs with zeta potentials near to zero.

**Characterization of unloaded NPs**

**The effect of the type of solvent and non-solvent**

As listed in table 2 (formulation 1-6), the NP size was dependent on the nature of the solvent confirming data obtained by other investigations where the use solvents with low dielectric constant or non-solvent, results in the larger final NPs (26). Using DW as non-solvent (formulations 1, 2, 3 and 8), and acetone produced the smallest NPs (formulation 8) and DMSO gave the largest NPs (formulation 1) which might be due to the rapid diffusion of acetone into DW (27). Therefore acetone was chosen as the solvent. Although NPs made by NVP were the smallest, but this solvent was not selected because of its toxicity in comparison with the acetone. By using PBS as non-solvent, increase in the NP size (comparing formulations 8 and 4) resulted in a complete precipitation after one day which might be due to the ionic interactions. Although using ethanol as non-solvent as reported by Bilati et al, produced PLGA NPs of 50 nm which were larger than water produced NPs (26), using ethanol in this study (formulation 5), resulted in NP size and zeta potential almost the same as formulation 8. Therefore DW was preferred to use as non-solvent and formulation 8 was chosen as the reference for other formulations in the article.

**The effect of PVA or PCLF Mw and concentration**

Formation of NPs in the absence of PVA (formulation 6) revealed that PVA is not a required component for nanoprecipitation method (Table 2). The size of NPs prepared in the presence of PVA (formulations 7 and 8) were smaller than those without PVA (formulation 6). Moreover, PVA22000 seemed to be more effective to form small NPs than PVA72000 apparently due its lower Mw (formulations 7 and 8). There was no considerable difference in PDI of all these three formulations. Therefore PVA22000 was used for all formulations. The effects of the PVA22000 concentration in DW on the NP size are given in figure 5. When the PVA concentration was increased, the NP size was decreased reaching to a mean size of 136.8±11.0 nm at PVA concentration of 0.15 w/v%, after which NP size increased by increasing the PVA concentration (28). Results for formulations 8-13 in table 2 reveal that higher the Mw of the PCLF, larger will be the related NPs. By using a certain concentration of PCLF, it was found that PCLF2000 and 1250 produced NPs larger than PCLF530 (compare formulations 8, 10 and 12, or formulations 9, 11 and 13) (26). Consistent with results of another report (28) findings of this study showed that the size became larger when concentration of PCLF in acetone was higher. Figure 6 shows that the NP size was increased dramatically by increase in PCLF2000 concentration. Figure 7 shows that the zeta potential decreased (becoming more negative) when the PCLF2000 concentrations decreased which may be attributed to the increase in the density of charge for smaller particles.

SEM images (Fig 8) demonstrated the spherical shape of the NPs with a relatively monodispersed size distribution which were in agreement with
Figure 4. Size distribution of the NPs by intensity.

Figure 5. Variation of the size of the NPs (formulation 8) prepared by using different concentrations of PVA22000 in DW (n=3).

Figure 6. Variations of the size of the NPs (formulation 8) prepared by different concentrations of PCLF2000 in acetone (n=3). The error bars of NP sizes are smaller than symbols.
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Figure 7. Variation of the zeta potential of the NPs (formulation 8) prepared by using different concentrations of PCLF2000 in acetone (n=3).

Figure 8. SEM images of NPs prepared by PCLF530 (A), PCLF1250 (B) and PCLF2000 (C), (formulations 9, 11 and 13, table 2, respectively).

Figure 9. Variation of the size of NPs (formulation 8) prepared by using different S/NS ratios (n=3).
Table 2. Size, PDI and zeta potential of unloaded NPs prepared by different formulations. The S/NS ratio for all formulations was 1:2 (n=3).

| Formulation No. | Solvent          | Non-solvent | PCLF Mw and concentration (w/v%) | PVA Mw and concentration (w/v%) | NP Size±SD (nm) | PDI     | Zeta potential (mV) |
|-----------------|------------------|-------------|----------------------------------|-------------------------------|-----------------|---------|---------------------|
| 1               | DMSO             | DW          | 2000,0.03                         | 22000,0.3                     | 364.9±23.6      | 0.3±0.0 | -9.1±2.1            |
| 2               | Methanol         | DW          | 2000,0.03                         | 22000,0.3                     | 233.5±17.3      | 0.1±0.0 | -14.5±9.3           |
| 3               | NVP              | DW          | 2000,0.6                          | 22000,0.3                     | 110.3±3.4       | 0.1±0.0 | -17.9±7.6           |
| 4               | Acetone          | PBS         | 2000,0.6                          | 22000,0.3                     | 211.5±5.6       | 0.1±0.0 | -33.2±17.1          |
| 5               | Acetone          | Ethanol     | 2000,0.6                          | 22000,0.3                     | 175.0±9.8       | 0.0±0.0 | -35.6±9.4           |
| 6               | Acetone          | DW          | 2000,0.6                          | 22000,0.3                     | -                | 0.1±0.0 | -44.5±9.5           |
| 7               | Acetone          | DW          | 1250,0.6                          | 22000,0.3                     | 197.0±4.3       | 0.1±0.0 | -33.1±7.2           |
| 8               | Acetone          | DW          | 530,0.6                           | 22000,0.3                     | 216.3±19.2      | 0.1±0.0 | -28.9±9.0           |
| 9               | Acetone          | DW          | 530,1                             | 22000,0.3                     | 176.0±7.2       | 0.1±0.0 | -28.1±3.2           |
| 10              | Acetone          | DW          | 1250,1                            | 22000,0.3                     | 185.9±29.2      | 0.1±0.0 | -32.2±6.6           |
| 11              | Acetone          | DW          | 1250                              | 22000,0.3                     | 117.3±10.1      | 0.1±0.0 | -52.7±4.3           |
| 12              | Acetone          | DW          | 530,0.6                           | 22000,0.3                     | 129.3±15.3      | 0.1±0.0 | -32.1±3.9           |

PDI, polydispersity index; SD, standard deviation; DW, deionized water; NVP, N-vinyl pyrrolidone.

Table 3. Size and PDI of NPs (formulation 8) prepared under different conditions; A: Stirring rate of 120 and 720 rpm, B: No stirring (0) and 20 minutes stirring after preparation, C: Dropwise addition (slow) and immediate addition (fast) of two phases (n=3)

|                | A: stirring rate (rpm) | B: stirring time (min) | C: Rate of the addition of acetone |
|----------------|------------------------|------------------------|-----------------------------------|
| NP size (nm)   | 120                    | 720                    | 0|20 | 198.6±22|163±7.2 |
| PDI            | 0.1±0.0                | 0.1±0.0                | 0.1±0.0                           | 0.1±0.0          | 0.1±0.0 |

The effect of S/NS ratio and process parameters

Figure 9 shows that the NP size did not change dramatically by increase in S/NS ratio (where other variables were selected the same as formulation 8), with an optimum ratio of 1:4 producing smallest NPs (141.2±11.9 nm). Similar results have been reported for formation of PLGA NPs (26).

As it may be found from table 3, smaller NPs were produced at high stirring rate (720 rpm) and the influence of stirring time after NP generation, was not significant on the NP size and when two phases were added slowly or in a dropwise manner, resulting NPs were larger than when the phases were added quickly or immediately, however difference in size of NP between these two methods, was not statistically significant (p>0.05).

Characterization of NPs after loading

NP size increased by increase in the initial concentration of Trb in DW (Fig 10). NPs prepared without PVA, were larger than those prepared with PVA22000. This phenomenon was not observed for the formulations using Trb at concentrations of about 0.05 or higher, probably because of the lower ability of PVA to reduce the NP size in the presence of high concentrations of Trb. According to table 4, when Trb concentration was 0.1% w/v, the NP size was 205.9±9.0 nm, zeta potential was -41.7±11.1 mV and DL and EE were 0.99±0.21 and 5.94±1.0, respectively (formulation 15) which in comparison with formulation 8, reveals a 20% increase in size after loading of Trb. A Comparison of formulations 20 and 21 with 8, reveals a 30 and 50% increase in the size respectively after loading of Dox. Such increase might indicate the entrapment of drug molecules in the NPs (30) but it could also arise from aggregation of the NPs. Zeta potential decreased after loading of the Trb or Dox, (formulations 14 to 21, table 4, compared with the unloaded NPs of formulation 8, table 2) which may be attributed to accumulation of drug molecules at NP surface. DL of Dox or Trb were quite low (increased by drug concentration), which
Table 4. Size, zeta potential, DL and EE of NPs prepared by different formulations (n=3). The solvent and non-solvent type, S/NS ratio and the PVA Mw and concentration, were the same as formulation 8 in table 2.

| Formulation No. | PCLF Mw & concentration (w/v%) | Drug concentration (w/v%) | NP size (nm) | Zeta potential (mV) | DL (w/w%) | EE (w/w%) |
|-----------------|---------------------------------|---------------------------|--------------|---------------------|-----------|-----------|
| Trb             |                                 |                           |              |                     |           |           |
| 14              | 2000, 0.6                        | 0.02                      | 179.0±3.1    | -35.2±2.8           | 0.21±0.02 | 6.3±1.1   |
| 15              | 2000, 0.6                        | 0.1                       | 205.9±9.0    | -41.7±11.1          | 0.99±0.21 | 5.94±1.0  |
| 16              | 2000, 1                          | 0.1                       | 288.6±12.5   | -27.3±6.6           | 1.01±0.58 | 10.10±1.25|
| 17              | 1250, 0.6                        | 0.1                       | 218.0±15.5   | -36.0±7.5           | 1.03±0.07 | 6.18±0.47 |
| 18              | 530, 0.6                         | 0.1                       | 121.6±24.7   | -40.6±6.0           | 0.32±0.10 | 1.92±0.60 |
| Dox             |                                 |                           |              |                     |           |           |
| 19              | 2000, 0.6                        | 0.02                      | 174.5±18.4   | -51.1±2.7           | 0.68±0.09 | 20.4±3.0  |
| 20              | 2000, 0.6                        | 0.1                       | 225.3±17.5   | -43.0±10.3          | 2.50±0.18 | 15.0±1.1  |
| 21              | 2000, 0.6                        | 0.2                       | 274.6±10     | -42.3±8.9           | 6.8±0.85  | 20.4±2.56 |

DL, drug loading; EE, entrapment efficiency of the drug.

Table 5. Size of NPs suspension of different formulations (in DW) after 0, 4 hrs and 1 week of preparation (n=3)

| Formulation No. | Time: 0 | 4 hrs | 1 week |
|-----------------|---------|-------|--------|
| 12              | 117.3±10.1 | 121.8±4.6 | 118.1±18.5 |
| 10              | 176.0±7.2  | 175±3.2  | 164.0±7.1  |
| 6               | 204.3±11.7 | 181.1±4.9 | 148.3±5.0  |
| 7               | 197.0±4.3  | 174.2±17.2 | 149.1±9.3  |
| 8               | 173.0±7.8  | 168.9±5.2 | 162.9±11.1 |
| 22              | 306.0±7.3  | 247.4±17.6 | 185.0±9.2  |
| 15              | 205.9±9.0  | 210.1±8.8 | 204.0±7.1  |
| 20              | 225.3±17.5 | 211.9±2.7 | 259.6±11.7 |

Formulation 22 was the same as formulation 8 but the concentration of PCLF in acetone was 2.5 w/w%.

According to the report (29) might be related to the large surface area of the NPs and the high water solubility of drugs accelerate drug loss into the water during NPs preparation. No significant difference was found in DL and EE between NPs prepared from PCLF2000 and 1250 (formulation 15 and 17) (p>0.05). However, the DL and EE related to the NPs prepared by PCLF530 (formulation 18) were small and significantly different from formulations 15 and 17 (p<0.05) which may be attributed to the PCLF530 lower Mw. When PCLF2000 concentration was increased from 0.6 to 1% w/v, the EE increased to 10% (formulations 15 and 16).

PCLF NPs suspended in DW, were stable even after one hour centrifugation at 14000 ×g, and 4°C. To precipitate the NPs, a g-force of 57438 ×g (26000 rpm) for 30 minutes was required. NP suspension showed almost no change in size for one week without considerable precipitate or aggregation especially when the drug was loaded (Table 5) and even after 6 months most of the NPs were still stable as suspension due to the surface charge of the NPs which is also reported previously (31). PBS accelerated NPs precipitation probably because of ion interactions on the surface charge.

The in vitro release (Fig 11) showed an initial burst release (more than 20%) in first 6 hrs, due to the fast release of the drug distributed close to the surface of the NPs. The release rate of Dox was shown to be more than Trb as 80% were released during 3 and 4 days respectively which may be related to the drug diffusion through the polymeric matrix (32) indicating the high inhibitory effect of the PCLF against release of the drug (29). Moreover, such sustained release could be attributed to the slow degradation of PCLF (13).

In summary very stable Dox loaded NPs with a size range of 174.5±18.4 to 344.5±31.7 nm...
can be prepared by an easy modification in nanoprecipitation method for NPs of different purposes. NPs could sustain drug release, owing to PCLF matrix and PCLF slow degradation. Although PCLF530 NPs were the smallest, they had lower DL than PCLF1250 and 2000 NPs.

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REFERENCES

1. Yang R, Shim WS, Cui FD, Cheng G, Han X, Jin QR, Kim DD, Chung SJ, Shim CK. Enhanced electrostatic interaction between chitosan-modified PLGA nanoparticle and tumor. Int J Pharm. 2009; 371: 142-7.
2. Matsumura Y MH. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumor tropic accumulation of proteins and antitumor agent SMANCS. Cancer Res. 1986; 46: 6387-92.
3. Kim SY, Ha JCH, Lee YM. Poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide)/poly(caprolactone) (PCL) amphiphilic block copolymeric nanospheres: II. Thermo-responsive drug release behaviors. J. Cont. Rel. 2000; 65: 345-58.
4. Park JS, Woo DG, Sun BK, Chung HM, Im SJ, Choi YM, Park K, Huh KM, Park KH. In vitro and in vivo test of PEG/PCL-based hydrogel scaffold for cell delivery application. J. Cont. Rel. 2007; 124: 51-59.
5. Zhou SH, Deng X, Yang H. Biodegradable poly(caprolactone)-poly(ethylene glycol) block copolymers: characterization and their use as drug carriers for a controlled delivery system. Biomaterials. 2003; 24:
3563-3570.

6. Huatan H, CJ, Attwood D. The microencapsulation of protein using a novel ternary blend based on poly (caprolactone). J. Microen. 1995; 12: 557-567.

7. Peng H, Ling J, Liu J, Zhu N, Ni X, Shen Zh. Controlled enzymatic degradation of poly(caprolactone)-based copolymers in the presence of porcine pancreatic lipase. Polym. Deg. Stab. 2010; 95: 643-650.

8. Wei X, Gong CY, Gou M, Fu SZ, Guo QF, Shi Sh, Luo F, Guo G, Qiu LY, Qian ZY. Biodegradable poly(caprolactone)-poly(ethylene glycol) copolymers as drug delivery system. Int. J. Pharm. 2009; 381: 1-18.

9. Gou M, Zheng L, Peng XY, Men K, Zheng XL, Zeng Sh, Shu G, Luo F, Zhao X, Chen LJ, Wei YQ, Qian ZY. Poly(caprolactone)-poly(ethylene glycol)-poly(caprolactone) (PCL-PEG-PCL) nanoparticles for honokiol delivery in vitro. Int. J. Pharm. 2009; 375: 170-176.

10. Shiin H, Ruhe PQ, Mikos AG, Jansen JA. In vivo bone and soft tissue response to injectable, biodegradable oligo(poly(ethylene glycol) fumarate) hydrogels. Biomaterials. 2003; 24: 3201-3211.

11. Dirk W. Grijpma QH, Jan Feijen. Preparation of biodegradable networks by photo-crosslinking lactide, e-caprolactone and trimethylene carbonate-based oligomers functionalized with fumacic acid monomethyl ester. Biomaterials. 2005; 26: 2795-2802.

12. Wang Sh, Lu L, Gruetzmacher J, Currier B, Yaszemski M. Synthesis and characterization of biodegradable and crosslinkable poly(e-caprolactone fumarate), poly(ethylene glycol fumarate), and their amphiipic copolymer. Biomaterials. 2006; 27: 832-841.

13. Wang Sh, Yaszemski M, Knight A, Gruetzmacher J, Windebank A, Lu L. Photo-crosslinked poly(e-caprolactone fumarate) networks for guided peripheral nerve regeneration: Material properties and preliminary biological evaluations. Acta Biomaterialia. 2009; 5: 1531-1542.

14. Sou K, Inenaga Sh, Takeoka Sh, Tsuchida E. Loading of curcumin into macrophages using lipid-based nanoparticles. Int. J. Pharm. Pharmaceutical Nanotechnology. 2008; 352: 287-293.

15. Paliwal R, Rai Sh, Vaidya B, Khati K, K. Goyal A, Mishra N, Mehta A, Vyas S. Effect of lipid core material on characteristics of solid lipid nanoparticles designed for oral lymphatic delivery. Nanomed. 2009; 5: 184-191.

16. Wang Sh, Yaszemski M, Gruetzmacher J, Lu L. Photo-crosslinked poly(caprolactone fumarate) networks: Roles of crystallinity and crosslinking density in determining mechanical properties. Polym. 2008; 49: 5692-5699.

17. Missirlis D, Tirelli N, Hubbell J. Amphiphilic hydrogel nanoparticles. Preparation, characterization, and preliminary assessment as new colloidal drug carriers. Langmuir. 2005; 21: 2605-2613.

18. Akbani H, Emanuele A, Attwood D. Effect of geometry on the erosion characteristics of polyanhydride matrices. Int J. Pharm. 1998; 160: 83-99.

19. Akbani H, Emanuele A, Attwood D. Effect of geometry on the erosion characteristics of polynhydride devices. Proceed Intern Symp Control Rel Bioact Mater. 1997; 24-30.

20. Peracchia MT FE, Desmaële D, Besnard M, Noël JP, Gomis JM, Appel M, d’Angelo J, Couvreur P. Stealth PEGylated polycyanoacrylate nanoparticles for intravenous administration and splenic targeting. J. Cont Rel. 1999; 60: 121-128.

21. Hakkarainen M, Albertsson AC. Heterogeneous biodegradation of poly-caprolactone-low molecular weight products and surface changes. Macromol Chem Phys. 2002; 203: 1357-1363.

22. Eldsater C, Erlendsson B, Renstad R, Albertsson AC, Karlsson S. The biodegradation of amorphous and crystalline regions in film-polycaprolactone). Polym. 2000; 41: 1297-1304.

23. Abou-Zeid DM, Muller RJ, Deckwer WD. Biodegradation of aliphatic homopolyster and aliphatic-aromatic copolysters by anaerobic microorganism. Biomacromolecules. 2004; 5: 1687-1697.

24. Harrison KL, Jenkins MJ. The effect of crystallinity and water absorption on the dynamic mechanical relaxation behaviour of polycaprolactone. Polym. Int. J. 2004; 53: 1298-1304.

25. Jenkins MJ, Harrison KL. The effect of molecular weight on the crystallization kinetics of polycaprolactone. Polym. Adv. Tech. 2006; 17: 474-478.

26. Bilati U, Allemann E, Doelker E. Development of a nanoprecipitation method intended for the entrapment of hydrophobic drugs into nanoparticles. Eur. J. Pharm. Sci. 2005; 24: 67-75.

27. Stainmesse S, Orecchioni AM, Nakache E. Modelling of an original process to obtain biocompatible polymeric nanospheres. Proc Sixth Congr Int Technol Pharm. 1992;39: 89-97.

28. Lamprecht A, Ubrich N, Hombreiro Perez M, Lehr CM, Hoffman M, Maincent P. Biodegradable monodisperse nanoparticles prepared by pressure homogenization emulsification. Int. J. Pharm. 1999; 184: 97-105.

29. Zhang J, Chen XG, Li YY, Liu CS. Self-assembled nanoparticles based on hydrophobically modified chitosan as carriers for doxorubicin. Nanomed. 2007; 3: 258-265.

30. Kim SY, Lee YM. Taxol-loaded block copolymer nanospheres composed of methoxy poly (ethylene glycol) and poly (caprolactone) as novel anticancer drug carriers. Biomaterials. 2001; 22: 1697-1704.

31. Muller RH, ed. Colloidal carriers for controlled drug delivery and targeting modification, characterization and in vivo distribution. FL, CRC Press, 1991; 57-97.

32. El-Malah Y, Nazzal S. Hydrophilic matrices: Application of Plackett–Burman screening design to model the effect of POLYOX–carbopol blends on drug release. Int. J. Pharm. 2006; 309: 163-170.