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Full Length Research Paper

Preparation and characterization of crosslinked and non-crosslinked polycaprolactone fumarate (PCLF) NPs as carriers for doxorubicin HCl

Narges Shokri[1], Hamid Akbari Javar[1,5]*, Shamileh Fouladdel[2], Ali Khalaj[3], Rasoul Dinarvand[1,4] and Ebrahim Azizi[2]

[1]Department of Pharmaceutics, Faculty of Pharmacy, Tehran University of Medical Sciences, 16 Azar street, Engelab Sq, P. O. Box 14155-6451, Tehran, Iran.
[2]Department of Pharmacology and Toxicology, Molecular research lab, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.
[3]Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.
[4]Medical Nanotechnology Research Centre, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.
[5]Department of Pharmacy, University of Malaya, Malaysia.

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Polycaprolactone fumarate (PCLF) is a biocompatible and biodegradable polyester which has been evaluated for tissue engineering. Hydrophobic PCLF nanoparticles (NPs) may be uptaken by reticule endotelial system rich organs and useful for treatment of related tumors. NPs of three PCLFs were produced through nanoprecipitation method. Crosslinked (CR) NPs were prepared using Benzyl peroxide (BPO) and N-vinyl pyrrolidone (NVP) under heating. Doxorubicin HCl (Dox) loaded NPs of PCLF530, 1250 and 2000 showed size of 149, 238 and 274 nm, respectively and zeta potential of about -40 mV, while their drug loadings (DL%) were 2.1, 6.0 and 6.8%, respectively. Dox was released from the NPs in 2 to 4 days in phosphate buffer saline, pH 7.4 at 37°C. Crosslinking of NPs could be completed at BPO/PCLF of 0.01 and NVP/PCLF of 0.1 at 40°C with no change in NP size. PCLF530 NPs possessed a higher CR% than two other NPs. The CR% was reduced in formulations without NVP and with NVP/PCLF of 0.8. Electron microscopic images revealed spherical (CR and not CR) NPs and core-shell structure. PCLF1250 NPs showed both more DL% and smaller size in relation to NPs of PCLF530 and 2000, respectively with a prolonged drug release profile and can be useful as a passive targeted drug delivery system. PCLF NPs could be CR (64.3%) through chemical crosslinking and consequently higher DL% (11.6%), longer drug release (6 days) and smaller size (188 nm) than not CR NPs.

Key words: Crosslinked PCLF NPs, doxorubicin HCl, nanoparticles.

INTRODUCTION

Polymeric NPs are promising vehicles for drug delivery. Of their important capabilities is their easier manipulation to prepare carriers with the objective of delivering the drugs to the specific target (Yang et al., 2009; Kim et al., 2000). Moreover, NPs can prolong blood circulation time of drugs and control the release rates of drugs in the tissues (Matsumura and Maeda, 1986). Poly (caprolactone) (PCL) is a hydrophobic biocompatible and biodegradable polyester which has been used for bio-resorbable sutures and scaffolds (Park et al., 2007) and for particulate drug delivery systems (Kim et al., 2000; Zhou et al., 2003). Its copolymer, poly (-caprolactone)–poly (ethylene glycol) copolymers has been synthesized and evaluated as a drug delivery system (Huatan et al., 1995). Fumaric acid has been used to synthesize polyesters suitable for crosslinking through fumarate double bonds like synthesized oligo (poly ethylene glycol-fumarate) and preparation of networks by photo-crosslinking of that (Dirk et al., 2005). Wang et al. (2006) have synthesized three molecular weight (Mw) of poly (caprolactone fumarate) (PCLF) from three Mw of PCLdiols

*Corresponding author. E-mail: akbarijo@sina.tums.ac.ir Tel: +98-021 66959090. Fax: +98 021 66461178.

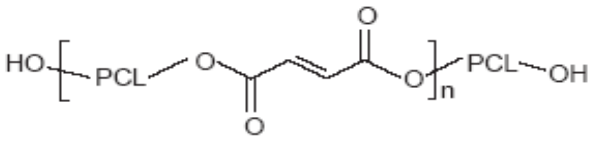

Figure 1. Schematic structure of PCLF.

(nominal Mw of 530, 1250 and 2000 g/mol) as precursors and used PCLF network for nerve regeneration (Wang et al., 2006, 2009). PCLF networks can be prepared through photo initiated crosslinking or thermal initiated crosslinking (chemical crosslinking) (Wang et al., 2006, 2009). Wang et al. (2008) have crosslinked (CR) the PCLFs by [bis(trimethyl benzoyl) phosphine oxide] (BAPO) (initiator) in methylene chloride (MC) under UV light which diminished the use of crosslinker. Dirk et al. (2005) have CR the PCLdiols oligomers by fumaric acid monoethyl ester in the presence of N,N-dicyclohexyl Carbodiimide and 4-dimethylamino pyridine at room temperature, and obtained networks with CR% of 68 to 86% for tissue engineering.

Fumarate not only makes the PCLF self-crosslinkable (crosslinking without incorporation of any crosslinker), but also enhances biodegradability of PCLF (Wang et al., 2008, 2009). PCLF nanoparticles (NPs) seems to encapsulate hydrophilic drugs better than simple PCL NPs, because of its polar fumarate segments. Such characteristic can be useful for adjusting drug loading (DL) and drug release behavior of PCLF NPs. Besides, hydrophobic PCLF NPs without a stealth shell are assumed to be uptaken by reticuloendothelial system (RES) reach organs in the body (Sou et al., 2008; Paliwal et al., 2009) and therefore be investigated as a passive targeted drug delivery system for treatment of lymphoma in our future work. According to available literature, preparation of PCLF NPs has not been reported until our work.

In our previous work (Shokri et al., 2011), the three Mw of PCLFs were synthesized (Wang et al., 2006) and identified and characterized by nuclear magnetic resonance (^{1}H-NMR) spectra, fourier transform infrared (FTIR) spectra, differential scanning calorimetry (DSC) and gel permeation chromatography (GPC). Degradation behavior of powdered PCLF in different media was determined during one month. Nanoprecipitation method was compared with microemulsion polymerization method and was preferred for preparation of PCLF NPs and several parameters affecting the NP preparation were investigated. The stability of PCLF NPs as an aqueous suspension was shown to be more than two weeks. In this study, doxorubicin HCl (Dox) was loaded into the NPs of three different PCLFs and the release profiles were obtained following by characterization and comparison of these NPs. Furthermore, CR PCLF NPs were prepared via a chemical crosslinking method (because of the sensitivity of Dox to the light). The impact of several factors on CR% and the effect of crosslinking on characteristics of the NPs were investigated beside the comparison of the CR NPs with not CR NPs.

MATERIALS AND METHODS

Fumaryl chloride, PCLdiols (nominal number-average molecular weight, M_n of 530, 1250 and 2000 g mol^{-1}), Doxorubicin HCl (Dox), Poly Vinyl Alcohol (PVA), N-Vinyl Pyrrolidone (NVP), Benzoyl Peroxide (BPO) and Dimethyl Toloidine (DMT) all were purchased from sigma-aldrich Co., Pottasium Carbonate (K_2CO_3), Dichloromethane or Methylene Chloride (MC), Acetone, Tetrahydrofuran (THF) and other chemicals were purchased from Merck, Germany and all were of synthesis grade.

Synthesis of PCLF

Synthesis and purification of PCLF was performed according to Wang et al. (2006). Briefly, three PCLdiols with nominal Mw of 530, 1250 and 2000 were employed to be sterified with fumaryl chloride in the presence of K_2CO_3 (proton scavenger), resulting in PCLF copolyesters (with different Mw of about 6000, 9000 and 11000, respectively (Table 1) which were named after their precursors as PCLF530, PCLF1250 and PCLF2000. Figure 1 shows the schematic structure of the PCLF. CR PCLF was obtained by adding a solution of BPO, DMT and NVP in MC (or in same NVP where MC was not used) to the PCLF and then placing of the mixture under 60°C for 15 to 50 min (Wang et al., 2009).

Preparation of PCLF NPs

PCLF NPs were prepared through nanoprecipitation method as reported in our previous paper (Sou et al., 2008; Peracchia at al., 1999). Briefly 2 ml of solution of PCLF in acetone (0.6w/v%) was poured into 8 ml stirring solution of PVA (0.3w/v%) in deionized water (DW) with or without Dox (0.2w/v%). Dox loaded NPs of three PCLFs and not copolymerized PCL(diol)2000 were obtained and compared in case of their size, zeta potential, DL and drug entrapment efficiency (EE). Moreover, for preparation of CR NPs, a solution of BPO, DMT and NVP in acetone (or in the same NVP when acetone was not used) and a separate solution of PCLF in acetone were poured in stirring DW (with or without Dox) simultaneously and the mixture was placed under 40°C and vigorous stirring for 30 to120 min. The evaporated amount of DW was continuously replaced with fresh DW during the heating. CR NPs were lyophilized (Lyotrap plus freeze dryer, LTE scientific co., UK) and then pressed onto a crystal in a thin layer form, to obtain their spectra by FTIR spectroscopy using a Nicolet FT-IR Magna 550 spectrophotometer.

Determination of CR%

Weighted CR PCLF or CR NPs (W_i: initial weight) were immersed in water for two days and dried by a vacuum oven. The dried samples were immersed in THF for two days and weighted (W_d: dried weight) after drying (Dirk et al., 2005). CR% was calculated as below:

$$CR\% = Wd/Wi \times 100$$

To calculate the heating time needed for completing the crosslinking, the time which the PCLF sample or NP formulation reached to a constant CR% was recorded in preliminary experiments. Each sample was tested in triplicate.

Table 1. Physical characteristics of PCLdiols and PCLFs.

Macromer or copolymer	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

Notes: M_n , number average molecular weight; M_W, weight average molecular weight; PDI, polydispersity index; T_m , melting point.

Determination of DL% and drug release profiles

Prepared NP suspension was filtered and separated using 0.1 µm membrane filter (PC type, Whatman, UK) and the concentration of unloaded drug in the filtrate solution was determined by absorbance of Dox in 485 nm using UV-VIS spectrophotometer (Jasco V-530, Jasco Co., Japan). Then DL% and EE% were calculated as below:

Amount of drug in NPs = Amount of drug added initially – Amount of drug in filtrate solution

DL (%) = Amount of drug in NPs / Amount of drug-loaded NPs × 100

EE (%) = Amount of drug in NPs / Amount of drug added initially × 100

Each sample was assayed in triplicate.

To determine drug release profile, 12 mg of NPs (collected over 0.1 µm membrane filter and washed two times with DW) was dispersed in 10 ml phosphate buffered saline (PBS), pH 7.4 which was poured into a dialysis bag. The bag was immersed in 30 ml sealed PBS, pH 7.4 and then placed in a shaker incubator (Heidolph incubator 1000, Heidolph Co., Germany) protected from light at 37°C under 70 rpm. 3 ml samples of the medium was taken at certain time intervals (every day) and 3 ml of fresh PBS was replaced every time for maintaining the sink condition. The concentration of Dox in the samples was determined by the UV absorbance at 485 nm. Each sample was examined in triplicate.

Characterization of the NPs

Each formulation was prepared in triplicate and the mean size ± standard deviation of them was reported. The effect of several variables of the method on NP characteristics was evaluated in order to adjust NP size, DL% and CR%. Every variable was changed while others were constant. The size, size distribution and zeta potential of the NPs were measured by laser light scattering (PCS) (Malvern Zetasizer, Nano-ZS series, Malvern co., UK). The shape of NPs and their size 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 removing water under reduced pressure.

RESULTS AND DISCUSSION

Synthesis and characterization of PCLFs and CR PCLFs

As reported before, the ^{1}H-NMR, FTIR, GPC and DSC spectra data of PCLFs were in accordance with the reported data by Wang et al. (2006). The Mw, polydispersity index (PDI) and melting point (T_m) of the PCLdiols and PCLFs are given in Table 1. According to FTIR spectra shown in Figure 4, the peak of fumarate double bond between 1500 and 2000 cm^{-1}, which was observed for PCLF1250 NPs, was disappeared for CR PCLF1250 NPs, indicating the consumption of double bonds during crosslinking process.

Using different concentrations of NVP and BPO and determining the residual of them (by UV absorbance at 230 and 235 nm respectively) after completion of cross-linking (constant CR%), revealed a nearly zero residual concentration of them when the NVP/PCLF was 0.1 and BPO/PCLF was 0.01. Therefore these ratios were considered as safe concentrations of NVP and BPO. Table 2 shows characteristics of CR PCLF samples. According to this table, it is observed that cross-linking of PCLF samples was accomplished efficiently at ratios of BPO/PCLF, DMT/PCLF and NVP/PCLF of 0.01, 0.01 and 0.2 respectively, at 60°C. The CR% of sample 4, 5 and 6 (80 to 94%) were higher than CR% of sample 1, 2 and 3 (66 to 80%) demonstrating that CR% of samples prepared by NVP and without any solvent, were more than CR% of samples prepared in the presence of both NVP and MC. Moreover, crosslinking of samples without MC, was completed quicker than crosslinking of samples with MC. Comparing CR% of samples 1 to 3, or samples 4 to 6 in Table 2, reveals that PCLF530 could bear much higher CR% in a shorter heating time than two other PCLFs because of its more fumarate segments and therefore more double bonds per copolymer chain.

Characterization of PCLF NPs

According to Table 3, NPs of PCLF2000, 1250 and 530 had sizes of 173±7, 176±7 and 117±10 nm, respectively with narrow size distribution which was previously reported for nanoprecipitation method (Bilati et al., 2005). Comparison of size of NP formulations 1, 3 and 5 (Table 3), made of PCLF2000, 1250 and 530 respectively, showed that increasing the Mw of PCLF, increased the NP size (Bilati et al., 2005). While NP formulations 1 and 2 were made of PCLF2000 with PCLF concentration of 0.6 and 1 w/v% respectively, NP formulations 3 and 4

Table 2. CR% of CR PCLF samples prepared with or without MC (n = 3).

Sample	PCLF Mw (0.6 w/v%)	BPO/PCLF	DMT/PCLF	NVP/PCLF	Solvent	60˚C (min)	CR%
1	530	0.01	0.01	0.2	MC	15	80.3±13.4
2	1250	0.01	0.01	0.2	MC	50	70±21.3
3	2000	0.01	0.01	0.2	MC	50	66.3±24.4
4	530	0.01	0.01	0.2	-	1	94.3±4.1
5	1250	0.01	0.01	0.2	-	15	82.3±10.6
6	2000	0.01	0.01	0.2	-	15	80±16.4

Notes: M_W, weight average molecular weight; BPO, Benzoyl peroxide; DMT, Dimethyl toluidine; NVP, N-vinyl pyrrolidone; CR%, crosslinked percent; MC, methylene chloride.

Table 3. Characteristics of unloaded NPs prepared with different formulations (n = 3). For all the NP formulations the S/NS volume ratio was 1:4, DW and PVA22000 (0.3w/v%) were used as non-solvent and stabilizer respectively.

Formulation no.	Solvent	PCLF Mw and concentration (w/v%)	NP Size±SD (nm)	PDI	Zeta potential (mV)
1	Acetone	2000,0.6	173±7	0.1±0.0	-31±5
2	Acetone	2000,1	216±19	0.1±0.0	-28±9
3	Acetone	1250,0.6	176±7	0.1±0.0	-28±3
4	Acetone	1250,1	185±29	0.1±0.0	-32±6
5	Acetone	530,0.6	117±10	0.1±0.0	-52±4
6	Acetone	530,1	129±15	0.1±0.0	-32±3
7	THF	2000,0.6	191±23	0.1±0.0	-28±1
8	NVP	2000,0.6	110±3	0.1±0.0	-17±7

Notes: M_W, weight average molecular weight; SD, Standard deviation; PDI, polydispersity index; THF, Tetra hydro furane; NVP, N-vinyl pyrolidone.

were made of PCLF1250 with PCLF concentration of 0.6 and 1 w/v% respectively and NP formulations 5 and 6 were made of PCLF530 with PCLF concentration of 0.6 and 1 w/v% respectively, it can be concluded that increasing the PCLF concentration, increased the NP size (Lamprecht et al., 1999). Using NVP as solvent resulted in smaller NPs in comparison with the NPs prepared by acetone as solvent. In contrast, using THF as solvent resulted in larger NPs in comparison with the NPs prepared by acetone as solvent. The PDI of all formulations was 0.1 indicating a narrow size distribution. The zeta potential was negative probably due to the carboxylic acid groups related to fumarate terminated copolymer chains. The zeta potential of NPs prepared with acetone was more negative than NPs prepared with NVP or THF.

Table 4 shows the Dox loaded NPs. As it can be seen from Table 4 increasing the initial concentration of Dox leading to an increase in NP size and DL% (formulation 9, 10 and 11). Such increase might indicate the entrapment of drug molecules in the NPs (Kim and Lee, 2001). Among the NP formulations 11, 12 and 13, NPs of 11 and 12 made of PCLF2000 and 1250, respectively, had higher DL% of about 6%. Such an observation and considering NP formulation 14, shows by increasing polymer Mw, DL% and EE% was increased. Also among these three formulations, NPs of 12 and 13 made of PCLF1250 and 530 respectively, had smaller size as 238±20 and 149±21 nm, respectively, which were suitable sizes as for a passive targeting system since most of the NPs have sizes under 200 nm. Therefore, although NPs of PCLF2000 (formulation 11) had the most EE%, NPs of PCLF1250 (formulation 12) possessed both desired characteristics as high DL% and small size. Such quite low DL% could be related to the large surface area of the NPs and the high water solubility of Dox accelerating drug loss into the water during NP preparation as has been reported (Zhang et al., 2007). Dox loaded NPs (Table 4) had more negative zeta potential than unloaded NPs probably because of the Dox molecules on the surface of the NPs (Table 3). The NPs were stable and did not show any change in mean size over one week in aqueous suspension due to the surface charge of the NPs which was reported previously (Muller, 1991). Also they were stable and recovered over lyophilizing.

Characterization of CR PCLF NPs

In Table 5, all NPs were made by simultaneous addition of a solution of 0.6w/v% PCLF in NVP or acetone and a

solution of BPO and DMT in NVP or acetone, into DW. According to Table 5, NPs of PCLF530 showed a higher CR% (81%, formulation 1) than CR% of NPs of PCLF1250 and 2000 (49 and 44.3%, formulation 2 and 3, respectively). Therefore not only the fact that PCLF530 can bear a higher CR% (mentioned in previous paragraphs) was confirmed, but also in comparison with the formulations 4 to 6 in Table 4, it can be concluded that PCLF NPs could have a lower CR% and need a longer heating time than PCLF copolymer samples, indicating the neutralization of the free radicals of crosslinking reaction by water molecules existed in NP preparation system. Comparison of the formulations 3, 4 and 5, demonstrated that for NPs placed under longer heating time (90 min, formulation 5), CR% was increased (66.3%) while a heating time more than 90 min did not lead to a more CR% (not shown in the table). Therefore 90 min could be an optimal heating time in such reaction. The very low CR% of the formulation 6 (5.6%) even after 120 min heating, shows that where NVP/PCLF was increased to 0.8, CR% fell down probably because of the diluting role of NVP. For the formulation 7 and 8, DMT was not used and for the formulation 8, NVP/PCLF was reduced to 0.1, but the CR% of them (65 and 64.3%, respectively) was not significantly changed in comparison with the CR% of NP formulation 5 (66.3%). Therefore presence of DMT was not necessary and a NVP/PCLF of 0.1 was chosen as an optimal ratio. According to formulations 9 to 15, the absence of NVP and the presence of acetone, reduced CR% dramatically (6.6 to 12%), demonstrating the significant role of NVP in such crosslinking reaction. Such a fact was mentioned by Dirk et al. (2005) as although fumaric acid has a high reactivity, its lower reactivity compared to acrylates, makes the use of comonomers like NVP, necessary. In case of NP formulations with increased heating time from 30 to 60 minutes (formulation 9 and 10, respectively), CR% was increased from 6.6 to 12%. While heating time more than 60 min did not lead to a more CR% (not shown in the table). CR% of NPs of PCLF530, 1250 and 2000 (formulations 10, 11 and 12, respectively) as 12, 7 and 9%, respectively, demonstrated again that PCLF530 NPs could be CR more than two other PCLFs because of its more double bonds. In the case of formulation 13 to 15, the BPO/PCLF and DMT/PCLF were increased from 0.01 to 0.04, but the CR% was not increased considerably (8 to 10.6%).

In Table 6, all NPs were made by simultaneous addition of a solution of 0.6w/v% PCLF2000 in NVP and a solution of BPO and DMT in NVP (except for formulations 1 and 4), into DW with or without Dox. Table 6 represents size, zeta potential and DL% of CR PCLF NPs. Formulation 1 was not treated with crosslinking agents and had a size of 133 nm. Formulations 2 and 3 were crosslinked during a heating time of 30 and 90 min resulting in CR% of 43 and 65.3% respectively, which proved again the effect of heating time on CR%. Formulations 2 and 3 had sizes of 109.6 and 136 nm respectively. Such a change in size implies that after 30 min of crosslinking, NP size was decreased (133 to 109 nm) which can be attributed to a primary shrinkage of the polymer during crosslinking and after 90 min of crosslinking, NP size was increased (109.6 to 136 nm) probably related to a following swelling. In addition, the ultimate size of CR NPs (136 nm, formulation 3) did not differ considerably from size of not CR NPs (133 nm, formulation 1). Zeta potential of these three mentioned formulations shows that crosslinking did not affect the zeta potential of the CR NPs and zeta potential of not CR NPs (-10 mV, formulation 1) was near to zeta potential of CR NPs (-17 and -11 mV, formulations 2 and 3, respectively). The zeta potentials of Dox loaded NPs (23 and 36 mV, formulations 4 and 5, respectively) were higher than unloaded NPs (-10 to -17 mV, formulations 1 to 3, respectively) indicating that DL could increase the surface charge of NPs. The latter fact was mentioned in previous paragraph, too. Obviously these zeta potentials which were related to NPs prepared by NVP, were lower than zeta potential of NPs prepared by acetone (Tables 3 and 4).

In case of NP formulation 4, no crosslinking agents were used and Dox was used during preparation of formulation with a concentration of 0.1 w/v% in water phase. Comparison of formulations 1 with 4, as mentioned before, shows that DL increased the NP size (133 and 141 nm, formulations 1 and 4, respectively). NP formulation 5 was the same as formulation 4 but crosslinking process was performed on it. Crosslinking in the presence of Dox not only increased the DL% dramatically (4.3 and 11.6%, formulations 4 and 5, respectively), but also increased the NP size (141 and 188 nm, formulations 4 and 5, respectively) while both facts could be due to the role of CR shell to inhibit drug washing out from NP matrix as was also found before (Dirk et al., 2005). CR% was not changed considerably in the presence of Dox (65.3 and 64.3%, formulations 3 and 5, respectively). PDI of 0.1 of all these formulations shows that crosslinking did not alter the monodispersity of NPs and the NPs were still narrow distributed after crosslinking.

SEM images of the NPs

SEM images as shown in Figures 2, 3, and 4, demonstrated narrowly dispersed and spherical shaped NPs and confirmed the PCS results in Table 2. No significant difference in the morphology was observed for NPs of PCLF with different Mw, whoever showing different sizes (Figures 2A, B and C). Figure 3 which presents images of CR NPs of PCLF 530, 1250 and 2000 (A, B and C, respectively), shows that CR NPs could gain a more spherical shape and an unexpected more mono-dispersity in size. Figure 4 is a close image of CR NPs of

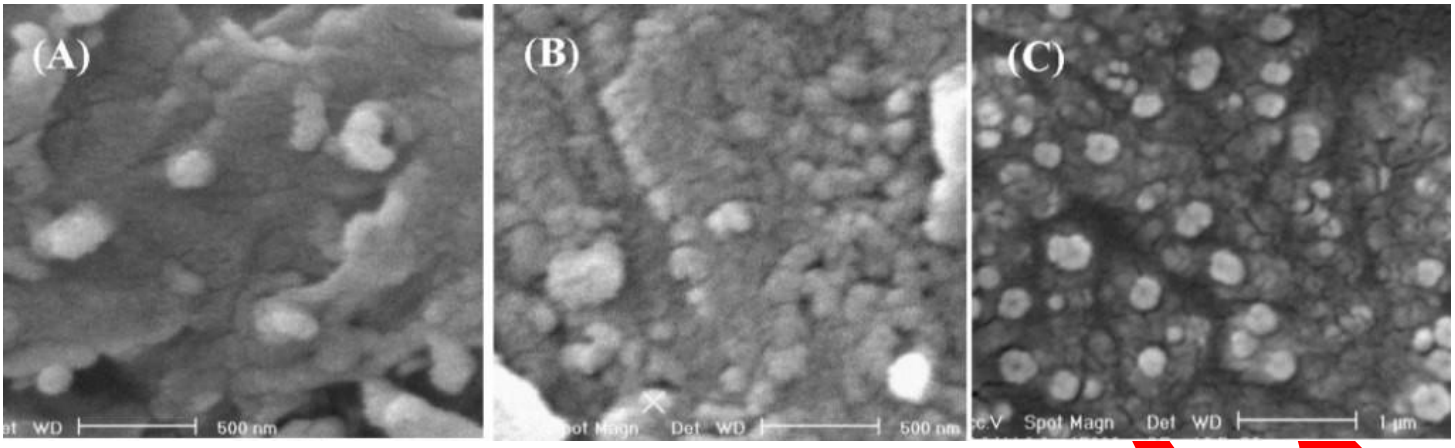

Figure 2. SEM images of NPs of PCLF530 (A), NPs of PCLF1250 (B) and NPs of PCLF2000 (C) (NP formulation 6, 4 and 2 respectively, Table 3).

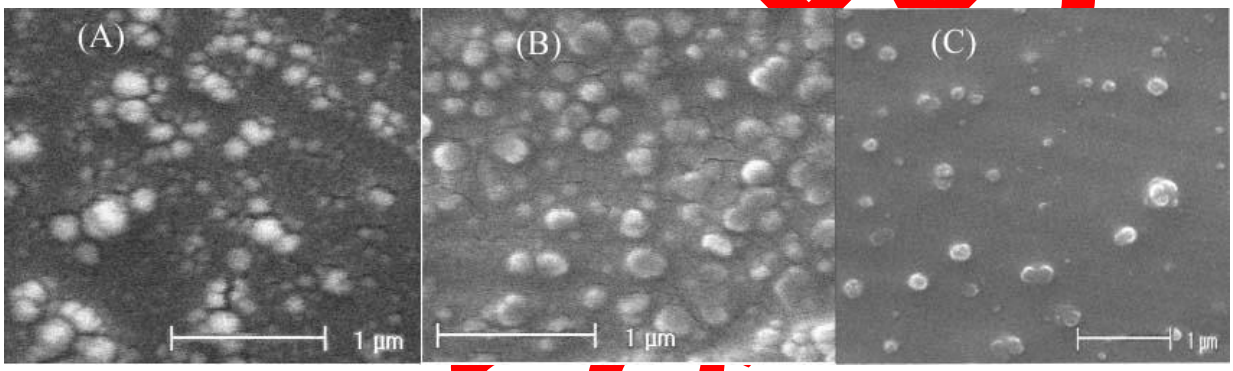

Figure 3. SEM images of CR NPs of PCLF530 (A), CR NPs of PCLF1250 (B) and CR NPs of PCLF2000 (C) (NP formulation 1, 2 and 3 respectively, Table 5). CR, Crosslinked.

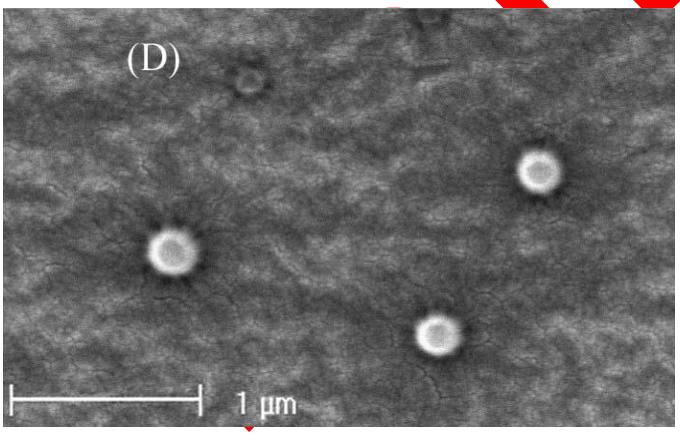

Figure 4. A close SEM image of CR NPs of PCLF1250 (D) to show the core/shell structure (NP formulation 2, Table 5). Notes: CR, Crosslinked.

PCLF1250, revealing a clear core-shell structure.

Drug release profiles

The *in vitro* release profile of Dox from NPs in PBS, shown in Figure 6, for all three types of NPs (made of PCLF530, 1250 and 2000) demonstrate an initial burst release (more than 20%) during the first 6 h of the release experiments, due to the fast release of the drug distributed close to the surface of the NPs. The cumulative percent of Dox released was shown to reach a plateau after 2 days for NPs of PCLF530 and after 4 days for NPs of PCLF1250 and 2000. Such sustained release could be attributed to the slow degradation of the PCLF in comparison with the other polyesters beside the high level of hydrophobicity of PCLF inhibiting the access of water to the polymer during the degrardation (Hakkarainen and Albertsson, 2002; Eldsater et al., 2000; Abou Zeid et al., 2004; Harrison and Jenkins, 2004; Jenkins and Harrison, 2006). Faster release related to NPs of PCLF530, could be as a result of the small size and large surface area of these NPs. Moreover, regardless to NPs of PCLF 530, the similarity of NPs of

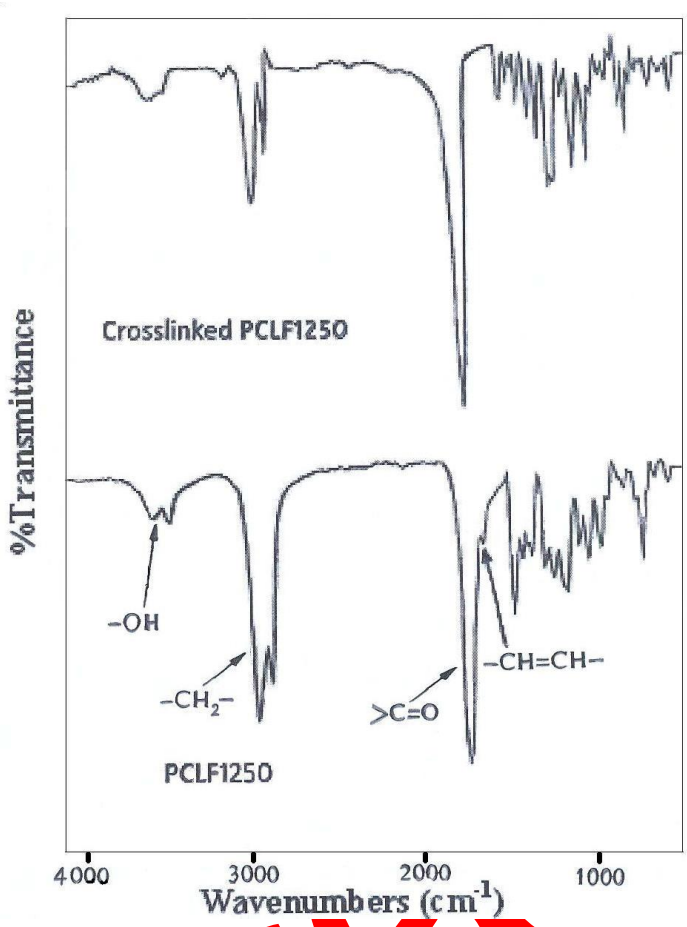

Figure 5. FTIR spectra of NPs of PCLF1250 and CR NPs of PCLF1250. Notes: CR, Crosslinked.

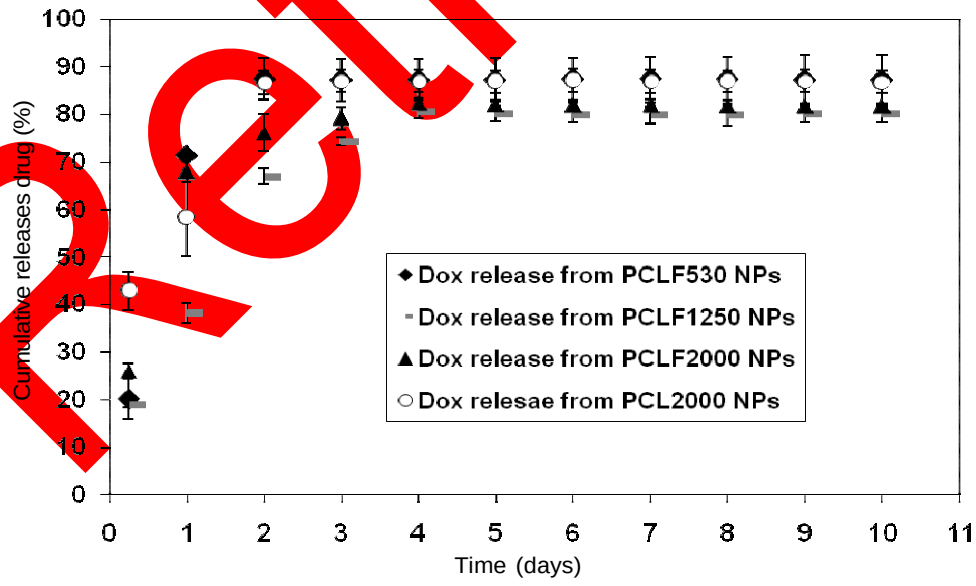

Figure 6. Release profile of Dox from NPs of formulations 11 to 14 (Table 3), in PBS, pH 7.4 at 37℃. Data are shown as cumulative percent of released Dox and as mean ± SD (n = 3). Notes: Dox, doxorubicin HCl; PBS, Phosphate buffer saline; SD, Standard deviation

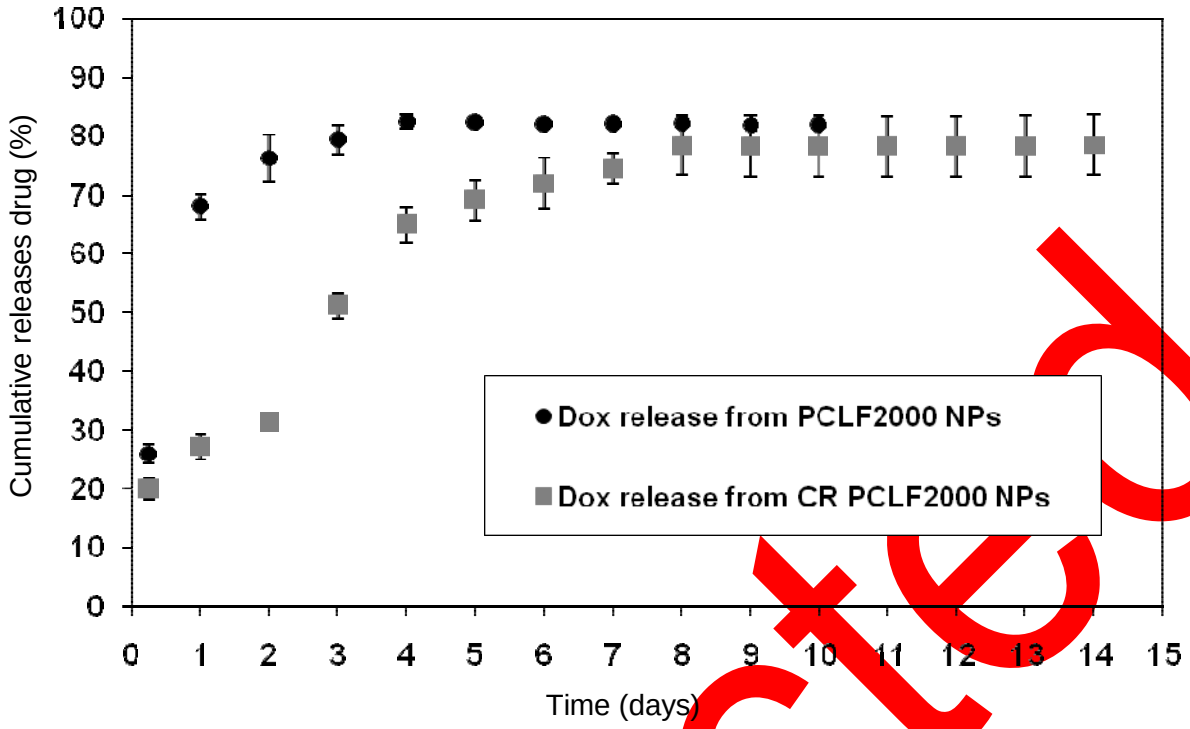

Figure 7. Release profile of Dox from NPs of formulations 11 (Table 3) and 5 (Table 6), in PBS, pH 7.4 at 37˚C. Data are shown as cumulative percent of released drug and as mean ± SD (n = 3). Notes: Dox, doxorubicin HCl; PBS, Phosphate buffer saline; SD, Standard deviation.

PCLF1250 and 2000, confirmed the reported data as Mw of polymer did not affect the drug release profile discriminately (El Malah and Nazzal, 2006). Dox release from not polymerized PCL NPs, was much quicker than the PCLF NPs and it reached to a maximum of 86.7% after 2 days showing relatively high SD. This could be because of the low Mw of the PCL(diol) 2000 macromer which apparently made unstable or fast desintegratable NPs. In Figure 7 it can be observed that the release rate of Dox from PCLF2000 NPs was more than Dox release from CR PCLF2000 NPs. PCLF2000 and CR PCLF2000 NPs released about 25.9 and 19.9% of their drug in first 6 h and 81.8 and 78.6% of their drug during 4 and 8 days, respectively. Such release profile was mainly due to the diffusion of the dissolved drug molecules through the polymeric matrix of the NPs, which was inhibited partiallyby CR shell in case of CR NPs.

Conclusion

PCLF copolymers with three different Mw (named PCLF530, PCLF1250 and PCLF2000) were synthesized from PCLdiols and fumaryl chloride. The PCLFs were used to prepare Dox loaded NPs through Nano-precipitation method. NP size dramatically increased when Mw and concentration of the PCLF was increased. PCLF530 made smaller NPs (149 nm) than two other PCLFs. PCLF1250 and 2000 NPs with size of 238 and 274 nm, had the more DL% as 6 and 6.8%, respectively. Based on these results of size and DL%, and also the PDI and zeta potential, PCLF1250 was seemed to be more suitable than two other PCLFs for preparation of a nanoparticulate drug delivery system. PCLF2000 NPs had the most EE% as 20.4%. The PDI of NP size were 0.1 indicating narrowly dispersed NPs. NPs of PCLdiol2000 (not copolymerized macromer) showed a low formulation reproducibility and could load the less Dox than NPs of PCLFs. Besides, PCL NPs had a low zeta potential which could decrease their stability as suspension. In contrast, Dox loaded PCLF NPs possessed a relatively high zeta potential (about -40 mV) which could provide their stability against aggregation and etc. Increasing the initial drug concentration caused a significant increase in the DL% and NP size. Moreover, increasing the PCLF Mw or concentration significantly increased the NP size.

CR PCLFs and CR NPs were prepared by BPO (initiator), DMT (accelerator) and NVP (comonomer) under the heating. FTIR spectra beside the CR%, proved the performance of crosslinking reaction. CR% or gel content was calculated by a sol extraction method to determine the level of crosslinking of samples. CR% was higher when NVP was used without any other solvent and PCLF530 showed a higher CR% in comparison with two other PCLFs. Best ratios of BPO/PCLF, DMT/PCLF

and NVP/PCLF were found to be 0.01, 0.01 and 0.1, respectively. CR% was minimized when NVP was more than needed. Increasing the heating time could increase CR% until the complete crosslinking (a constant CR%) and a 90 min heating was found to be enough for a complete crosslinking reaction. Complete crosslinking (without DL) did not affect NP size and PDI but increased the DL% to 11.6% and subsequently increased the NP size to 188 nm. NPs prepared by NVP as solvent instead of acetone, showed a lower zeta potential of -10 to -17 mV which after DL was increased to -23 and -36 mV.

SEM images of PCLF NPs and CR PCLF NPs confirming the PCS results, presented spherical narrowly dispersed NPs which were better shaped and with lower PDI in case of CR NPs than not CR NPs. A closed image of CR PCLF1250 NPs, revealed a core-shell structure as the result of crosslinking process. Dox was released from PCLF NPs, with a burst release of about 20% after 6 h and about 80% release during 2 or 4 days. Dox release from PCLdiol2000 NPs was much more rapid with a high SD. Dox release from CR PCLF2000 NPs, showed a slightly lower burst release after 6 h in comparison with Dox release from PCLF2000 NPs and also showed a slower release of 78.6% in 8 days. Overall, PCLF NPs could be prepared by nanoprecipitation method, with suitable size, PDI, zeta potential and DL% for a nano-particulate drug delivery system. PCLF NPs could be CR successfully resulting in NPs with suitable size, increased DL%, improved spherical shape and a sustained drug release profile.

Abbreviations: PCL, Poly caprolactone; **PCLF,** poly caprolactone fumarate; **NP,** nanoparticle; **DW,** deionized water; **PBS,** phosphate buffered saline; **Dox,** doxorubicin HCl; **CR,** crosslinked; **PVA,** polyvinyl alcohol; **BPO,** benzoyl peroxide; **DMT,** dimethyl toluidine; **NVP,** N-vinyl pyrrolidone; **MC,** methylene chloride; **THF,** tetra hydro furan; **DL,** drug loading; **EE,** drug entrapment efficiency; **Mw,** molecular weight; **Tm,** melting temperature; **PDI,** polydispersity index; **SD,** standard deviation; **RES,** reticuloendotelial system; **SEM,** scanning electron microscopy.

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