J Mater Sci: Mater Med (2009) 20:821–831
DOI 10.1007/s10856-008-3637-5

# Novel self-assembled amphiphilic poly(ε-caprolactone)-grafted-poly(vinyl alcohol) nanoparticles: hydrophobic and hydrophilic drugs carrier nanoparticles

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Received: 16 July 2008 / Accepted: 20 October 2008 / Published online: 20 November 2008


**Abstract** In the present study, we have aimed to produce nanoparticles (NPs) possessing the capability of carrying both of the hydrophobic and hydrophilic drugs and reveal significant release for both drug types. Poly(ε-caprolactone) (PCL) grafted poly(vinyl alcohol) (PVA) copolymer (PCL-g-PVA) has been prepared and shaped in nano-particulate form to be adequate for carrying the drugs. Stannous octoate ($Sn(II)Oct_2$) was used to catalyze PVA and ε-caprolactone monomer to chemically bond. Moreover, this catalyst enhanced side chain polymerization reaction for the utilized ε-caprolactone monomer to form poly(ε-caprolactone) (PCL). The formed PCL was attached as branches with PVA backbone. $^1H$ NMR has confirmed formation of PCL and grafting of PVA by this new polymer. Moreover, the vibration modes in the functional groups of PCL-g-PVA have been detected by FT-IR. The thermal alteration in the grafted polymer was checked by TGA analysis. The successfully synthesized grafted copolymer was able to self-aggregate into NPs by direct dialysis method. The size, morphology and charges associated with the obtained NPs were analyzed by DLS, TEM and ELS, respectively. PCL-g-PVA NPs were investigated as drug carrier models for hydrophobic and hydrophilic anti cancer drugs; *paclitaxel* and *doxorubicin*. In vitro drug release experiments were conducted; the loaded NPs reveal continuous and sustained release form for both drugs, up to 20 and 15 days for *paclitaxel* and *doxorubicin*, respectively. However, in a case of using pure drugs only, both drugs completely released within 1–2 h. The overall obtained results strongly recommend the use these novel NPs in future drug delivery systems.

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## 1 Introduction

In recent years, capability of PVA has been actively explored in the field of nanomedicine and nanobiotechnology such as nanoparticles and nanofibers, which serves the purpose of drug delivery system or wound dressing agents [1, 2]. Modification of PVA by fibronectin protein leads to promote fibroblast adhesion [3]. PVA has been also frequently utilized for cell culture attachment as hydrogels and bioartificial pancreas [4, 5]. Recently, PVA has been cleared by the United States Food and Drug Administration (FDA) as a safe component for coatings and adhesives that come into contact with fatty foods [6]. However, there is restriction for using pure PVA in the

drug delivery fields due to its high hydrophilicity. To overcome this dilemma, PVA has to be chemically modified in a way decreasing the hydrophilic nature. One interesting chemical treatment method is exploiting some hydrophobic polymers to block the hydrophilic sites in the PVA chain. For instance, some researchers have invoked poly(lactic-co-glycolic acid) for this task, PLGA-g-PVA was produced [7].

Poly(caprolactone) PCL is hydrophobic, biocompatible, biodegradable and non-toxic polyesters. In biomedical applications, it was found that it is completely biodegradable after interaction with bacterial lipases which suggests its use as biomaterial [8]. Moreover, PCL has been approved by (FDA) for component in contraceptive implants [9]. PCL does have high hydrophobic feature; some researchers have modified PCL by dextran in order to decrease its hydrophobicity [10]. Decreasing the hydrophobic nature of PCL by grafting it with hydrophilic dextran resulted in increase cell attachment to the nanofibrous mats [11]. PCL-g-dextran was able to form NPs which expressed low or non toxic effect on cancer cells [12]. It is noteworthy mentioning that PVA has been grafted by poly(ε-caprolactone) via ring opening solution polymerization process but final product in the form of NPs were not addressed [13].

In the present study PCL and PVA as biologically non invasive and non immunogenic in nature polymers were exploited to produce PCL-grafted-PVA in nano particulate form to be adequate for drug carrying. The synthesized NPs have been loaded by two different anti-cancer drugs; *paclitaxel* and *doxorubicin*. In vitro drug release revealed satisfactory results for both drugs.

## 2 Experimental work

### 2.1 Materials

Poly(vinyl alcohol) (PVA) (MW = 65000) was obtained from Dong Yang Chem., Co. Korea. While, ε-caprolactone (ε-CL) and stannous 2-ethyl hexanoate ($Sn(oct)_2$) were purchased from Sigma-Aldrich Inc. USA. Dialysis tubing's (MWCO 12000-14000, Membrane Filtration Products Inc., USA) was used in the dialysis process. Paclitaxel (Aldrich Co., USA) and doxorubicin hydrochloride (≥98% purity, Sigma-Aldrich Inc., USA.) were utilized as hydrophobic and hydrophilic drug models, respectively. Phosphate buffer saline solution (PBS) (Aldrich Co., USA) with concentration of 0.1 M and pH of 7.4 was used in the drug release experiments. Dimethyl sulfoxide (DMSO) and toluene solvents (both were obtained from Showa, Japan,) were refluxed over $CaH_2$ and stored under nitrogen atmosphere before use.

### 2.2 Procedure

#### 2.2.1 Synthesis of copolymer

Synthesis of grafted polymer was performed according to previously reported method [13]. Briefly a mixture of dried PVA; 1.0 g and DMSO; 20 g was charged in a four-necked flask equipped with a stirrer, a nitrogen inlet, an outlet, and a thermometer, then further stirred at 60°C until the PVA was completely dissolved. Subsequently, 1:50 catalyst stannous 2-ethyl hexanoate [$Sn(oct)_2$] in toluene based on the weight of the reactant were added slowly via glass syringe drop wise into a flask containing a pre-weighted amount of PVA. This reaction mixture was allowed to react for 30 min in order to activate the hydroxyl groups of PVA by catalytic effect under continuous nitrogen environment. After the activation process, 20 g of ε-caprolactone monomers (previously nitrogen purged for 24 h) were added to the four neck flask. Then, the reaction mixture was continuously stirred at 100°C under a nitrogen atmosphere for 12, 24 and 48 h, respectively. The produced copolymers batches were extracted by precipitation in an excess amount of cold ether which also dissolves the unreacted caprolactone monomers. The isolated products were carefully washed with deionized water to remove the unreacted PVA as a precautionary measure. Further on resulting products were dried under vacuum in presence of $P_2O_5$ at 80°C for at least 2 days to remove residual solvents.

#### 2.2.2 Formulation of PCL-g-PVA nanoparticles

Shaping of the obtained copolymer into the form of NPs was achieved by the following way: 40 mg of grafted polymer was dissolved in 10 g of DMSO, loaded in dialysis tubing and dialyzed against triple-distilled water at room temperature. After loading, the distilled water was exchanged every 1 h within the first 3 h in order to remove the bulk of DMSO from dialysis tube. Hereafter, distill water exchange was done slowly for every 3 h for an additional 45 h for complete self-assembly of NPs. After dialysis process, the obtained products were sonicated for 5 min. Further on samples were analyzed for future investigations.

#### 2.2.3 Drug loading experiments

The in vitro drug release experiments were carried out in two different ways. As *paclitaxel* and *doxorubicin* are different in nature; hydrophobic and hydrophilic drugs, so, they were incorporated in the core and shell of the NPs. Therefore, loading of *paclitaxel* drug has been achieved during performing the self-assembly process. However,

loading of *doxorubicin* has been carried out after the self-assembly process.

Typically, encapsulation *paclitaxel* drug inside the core of NPs has been achieved as follow: 40 mg of PCL-g-PVA copolymer were dissolved in 9 g of DMSO and dialyzed against triple-distilled water using dialysis tubing at room temperature. The distilled water was exchanged every 1 h for the first 3 h and then every 3 h for an additional 21 h. After passing the 24 h, the slurry in the dialysis bag which can be considered semi-self-assembled NPs was mixed with 1 mg of paclitaxel dissolved in 1 ml of DMSO and kept under stirring for 12 h. Further, this new mixture was again dialyzed; triple-distilled water exchange was done every 3 h for 24 h. Further on samples were analyzed.

For *doxorubicin*, drug loading was achieved by adding 1 mg drug dissolved in 1 ml of water to 10 ml of the completely self assembled NPs recovered after dialysis as aforementioned in Sect. 2.2.2. This solution was simply stirred for 12 h, so, the drug was attached with the NPs shell. Further on, the coated NPs with drug were analyzed for other investigations. It is noteworthy mentioning that in both cases the loaded NPs slurries were used in the drug release experiments, however, isolating the loaded NPs is not difficult task; it can be done by centrifugation.

### 2.2.4 *Drug release experiments*

For the drug release experiments, 40 mg of the prepared NPs slurry were loaded by either *paclitaxel* or *doxorubicin* as aforementioned. This slurry was filled in dialysis tubing and placed in 150 ml specially designed glass bottle taking care of evaporation and equipped with controlled temperature system. Ninety ml autoclaved PBS solution was placed in the glass bottle sinking the dialysis tubing; the media was stirred (100 rpm) at 37 ± 2°C. To check the amount of the released drug, the first samples were withdrawn within short times (i.e. after 1, 2, 4, 8, 16 and 24 h), later, one sample was taken everyday. In every sampling time, an equal amount of the pipetted out solution was replaced with fresh PBS in order to avoid saturation.

To safely estimate the drug concentration in the PBS solution, the absorbance intensities of the utilized drugs have been measured for many drug/PBS solutions. In a case of the *paclitaxel*, the UV absorbance spectra for different concentrations drug solutions start from 0.0049 until 7.5 mg/l were measured within the range of 100–260 nm. Figure 1 reveals the obtained results. As shown in this figure, the absorbance curves have maximum values at almost 209 nm. Moreover, the maximum measured absorbance intensities were linearly increased with increasing the drug concentration within two ranges as shown in Fig. 2a; low concentration range (from blank to 0.4 mg/l) and high concentration one (from 0.4 to 7.5 mg/l).

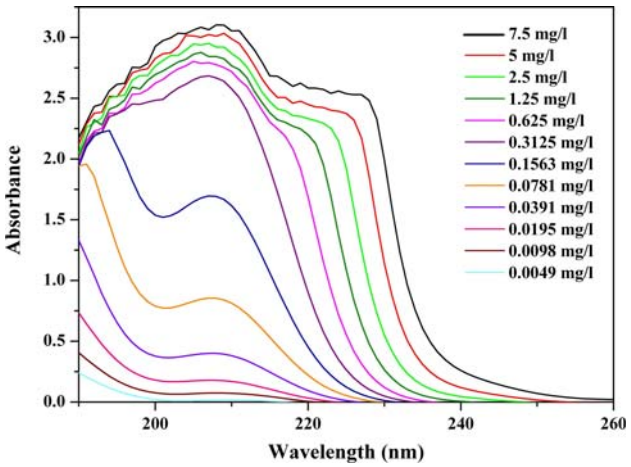



**Fig. 1** The relationships between the absorbance intensity and the wavelength for many paclitaxel drug/PBS solutions

Figure 2b and c shows the relationships between the drug concentration and the measured absorbance at the low and high concentration ranges, respectively. As shown in these figures, the absorbance at 209 nm varies linearly with the drug concentration in good linear models. Statistical analyses of these two curves indicated high accuracy of the exploited linear models since the coefficient of determination; $R^2$ of these models were 0.997 and 0.989 for Fig. 2b and c, respectively, which reveals good precision and reproducibility of these calibration curves. However, a single linear model could not accurately represent the whole data points since the mathematical errors would be unacceptable as can be concluded from Fig. 2a; the coefficient of determination of a single linear model was 0.564. It is noteworthy mentioning that the corresponding standard deviation values for all the data points are very small as shown in the insets of Fig. 2b and c. Therefore, the concentration of the drug in the release experiments has been conducted by measuring the absorbance at 209 nm and applying in Fig. 2b or c to estimate the corresponding drug concentration.

By the same fashion, Fig. 3 shows the absorbance spectra for many PBS/*doxorubicin* drug solutions (from 0.0391 to 10 mg/l) within wavelength range of 360–640 nm. As shown in this graph, maximum absorbance was obtained at 486 nm for all drug concentrations. It is noteworthy mentioning that this drug reveals very good linearity compared with *paclitaxel* since a single linear model was enough to represent all the data points with very small mathematical errors as shown in Fig. 4; the coefficient of determination of this single linear model was 0.998. The standard deviation values were placed in the inset of this figure.

All data originating from drug release experiments were statistically analyzed using Student's *t*-test and one-way

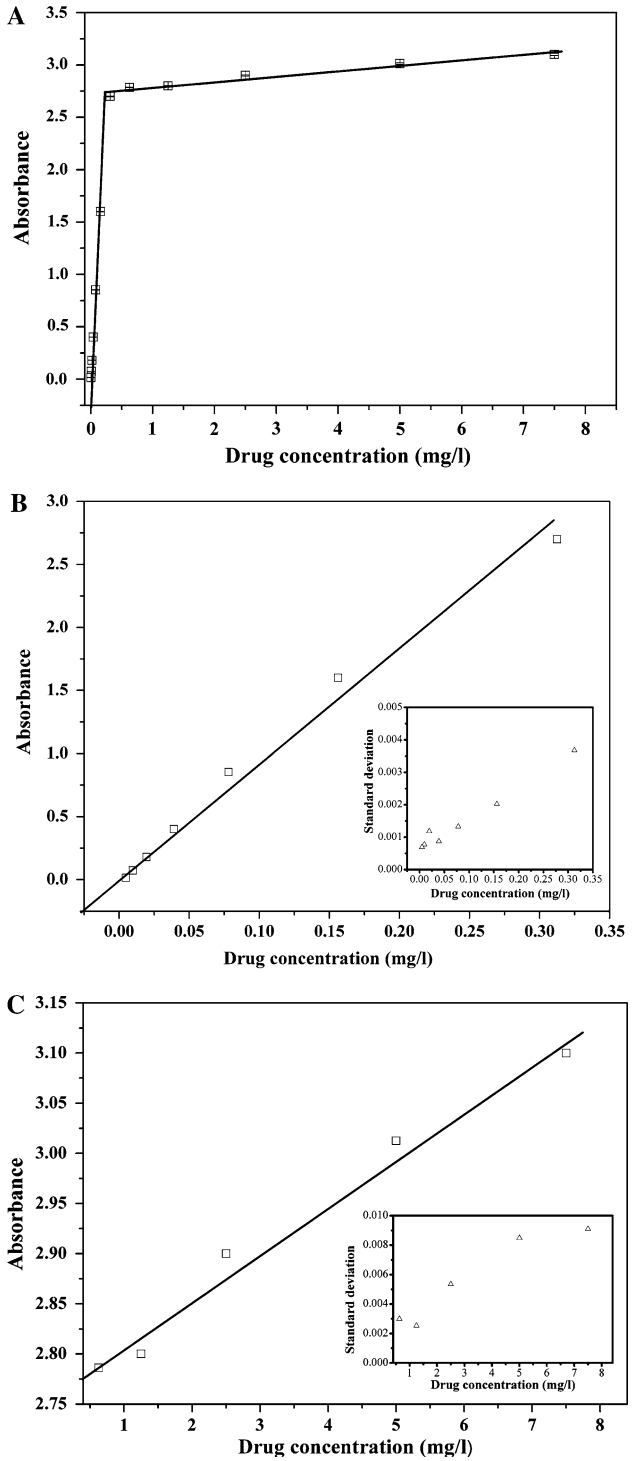



**Fig. 2** The relationship between the drug concentration in PBS solution and the absorbance at wavelength of 209 nm: all utilized solutions concentrations (**a**), low concentrations (**b**) and high concentrations (**c**)

ANOVA tables since three test samples have been utilized, the difference was significant when $P < 0.05$. All these statistical analyses for finding out the release pattern were performed via MATLAB 6.2.

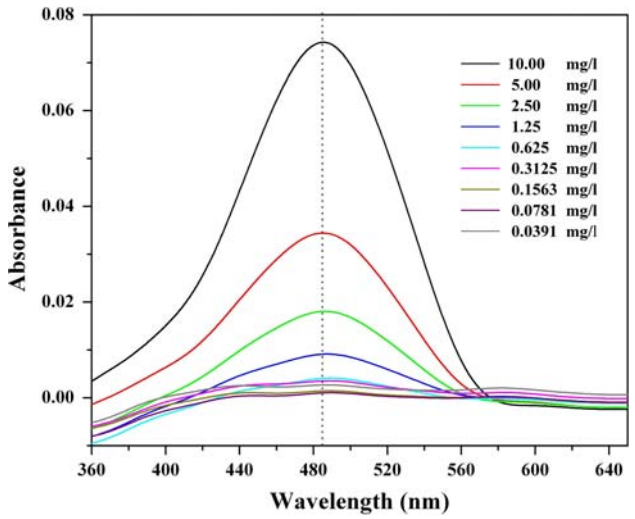



**Fig. 3** The relationships between the absorbance intensity and the wavelength for many doxorubicin drug/PBS solutions

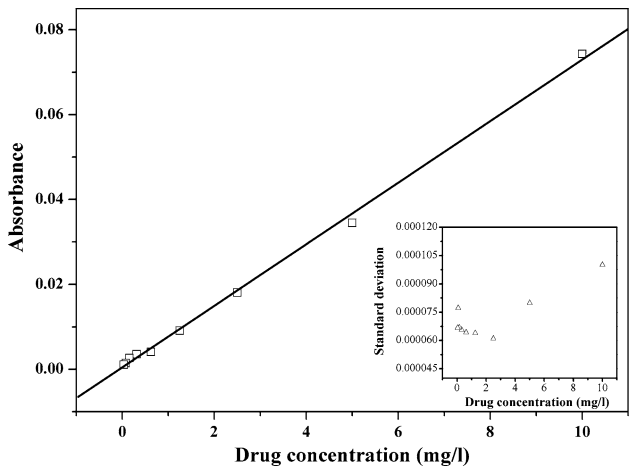



**Fig. 4** The relationship between the drug concentration in PBS solution and the absorbance at wavelength of 486 nm: all utilized solutions concentrations

## 2.3 Characterization

Chemical structure of PCL-g-PVA was characterized by $^1$H NMR spectra recorded with a JNM-Ex 400-NMR spectrometer (JEOL Co., Japan, operated at 400 MHz). Samples were made (prepared) by 4% (w/v) solution in deuterated dimethyl sulfoxide (DMSO-$d_6$) using tetramethylsilane (TMS) as an internal standard. To identify the vibration in functional groups of grafted polymer; FT-IR analysis was done using a Varian FTS 1000 spectrometer scimitar series (Varian Inc, Co., Australia). The samples were made by pelletising the grafted polymer powder with potassium bromide. The thermal stability of the synthesized amphiphilic PCL-g-PVA was carried out with a (Pyris TGA Perkin Elmer Inc., USA) by heating in nitrogen

atmosphere from 25 to 700°C with the heating rate was 10°C/min. Heating was followed under a continuous nitrogen purge of 20 ml/min. Hydrodynamic particles size and size distribution were determined by dynamic light scattering (DLS) (Malvern System 4700 instrument, Otsuka Electronics Co., USA) equipped with vertically polarized light supplied by an argon-ion laser (Cyonics) operated at 20 mW. All experiments were performed at room temperature with measuring angle of 90° to the incident beam. Surface charge $\zeta$-potential of the NPs was determined with electrophoretic light scattering (ELS) measurement by (ELS 8000/6000 Otsuka Electronics Co., Japan) at room temperature with a measuring angle of 20° when compared to the incident beam. Samples were sonicated in an ultra-sonicator bath for one minute prior to analyses. NPs prepared were observed by transmission electron microscopy (BIO-TEM, SN-3000, Hitachi, Japan) operated at an accelerating voltage of 80 kV. The samples for TEM measurements were prepared by dipping the TEM copper grid in (1 mg/ml) an aqueous dispersion of NPs. For staining, a drop of 2% phosphotungstic acid (PTA) solution was pipetted out by micropipette on freshly dipped TEM copper grid. The extra solution was removed by using a Kimwipes and the grid was allowed to dry overnight under vacuum. Before loading, samples were sonicated in an ultra-sonicator bath for 1 min. All samples required for DLS and ELS were used without any filtration and were done in triplicates. Drug release measurements were performed by using HP 8453 UV–Visible spectroscopy system, Germany. The optical density (OD) was measured at wavelengths of 209 and 486 nm for *paclitaxel* and *doxorubicin*, respectively. The spectra obtained were analyzed by HP ChemiStation software 5890 series.

## 3 Results and discussion

### 3.1 Synthesized grafted polymer characterization

As aforementioned in Sect. 2.1, the reactants were PVA and $\varepsilon$-caprolactone monomer. It is noteworthy mentioning that the used catalyst; ($Sn(II)Oct_2$) does have the ability of opening the $\varepsilon$-caprolactone ring which attached chemically with PVA chain. Also, this compound catalyzes side chain polymerization reaction to form ploy(caprolactone) branches on the PVA backbone [13]. To form NPs from a grafted polymer the specific ratio of macro initiator PVA and caprolactone monomer are very crucial to obtain the desired hydrophobic and hydrophilic balance in amphiphilic grafted polymer. In order to achieve this goal, a fixed amount of feed ratio of PVA, caprolactone monomer and catalyst were used and only reaction time was varied as 12, 24 and 48 h as indicated in Table 1. The formation of PCL-g-PVA at this stage was characterized by $^1$H NMR. The spectral feature as shown in Fig. 5 pictured the well-defined chemistry of the graft co-polymer. The distinctive feature of PCL-g-PVA in this figure shows the characteristic peaks of PCL at $\delta$ 4.12–4.02 [–(CO)–$CH_2CH_2CH_2CH_2CH_2O$, the fifth methylene group connected to carbonyl of repeating unit of PCL], 3.70–3.62 [–(CO)–$CH_2CH_2CH_2CH_2CH_2OH$, end group of PCL], 2.35–2.24 [–(CO)–$CH_2CH_2CH_2CH_2CH_2O$, the first methylene group connected to carbonyl of repeating unit of PCL], 1.72–1.56 [–(CO)–$CH_2CH_2CH_2CH_2CH_2O$, the second and fourth methylene groups connected to carbonyl of repeating unit of PCL], 1.42–1.33 ppm [–(CO)–$CH_2CH_2CH_2CH_2CH_2O$, the third methylene group connected to carbonyl of repeating unit of PCL]. The degree of polymerization (DP) and degree of substitution (DP) of the graft copolymers were determined by end group analysis of the PCL chains [13, 14]. In this regard Table 1 represents the DP and DS for PCL content on PVA chains.

On the other hand, the FT-IR spectrum of amphiphilic PCL-g-PVA copolymer was studied for its grafted functionality. We have utilized pure PCL (MW = 80,000, Aldrich) to confirm occurring polymerization reaction during the grafting process. Figure 6 represents the FT-IR spectrum of pure PCL, pure PVA and amphiphilic PCL-g-PVA copolymer. As shown in this figure, the spectrum of the PCL-g-PVA does have all the peaks present in both PCL and PVA spectra. In more details, the grafted polymer shows absorption band at 3433 $cm^{-1}$ representing vibration mode in OH group. This peak is also appears in PVA, so, one can say that this peak represents the unreacted OH groups in the PVA backbone in the grafted polymer. Also, it can reveal the end terminal of PCL branch. At 1720 $cm^{-1}$ a strong peak appears in the grafted polymer, this peak represents C=O carbonyl ester stretching. Sharpness of this peak confirms occurring the grafting as well as the side chain polymerization reaction since this peak is relatively weak in case of pure PCL which means more ester bonds in the grafted polymer than PCL. A new

**Table 1** Characterization of PCL-g-PVA determined by $^1$H NMR spectra taken in (DMSO-$d_6$)

| Sample | Reaction time | Degree of polymerization | Degree of substitution | PCL content (wt%) | Catalyst/caprolactone ratio |
|---|---|---|---|---|---|
| PVA-PCL-1 | 12 | 2.1 | 0.15 | 43 | 1:50 |
| PVA-PCL-2 | 24 | 3.5 | 0.17 | 62 | 1:50 |
| PCA-PCL-3 | 48 | 4.2 | 0.41 | 69 | 1:50 |

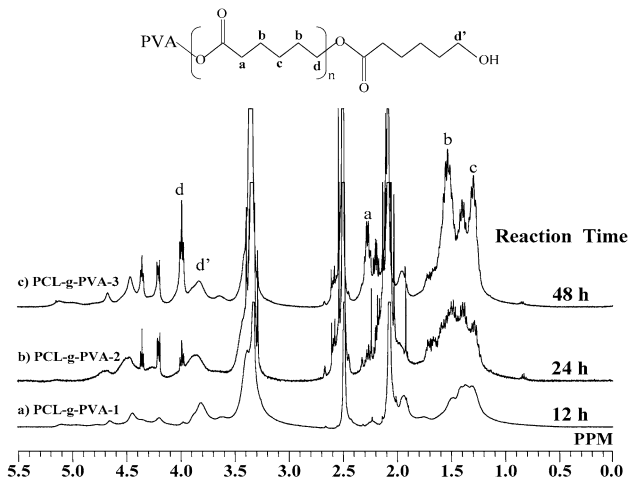



**Fig. 5** $^{1}$H NMR spectra of different grafted copolymer batches with reaction times; 12 h, PCL-g-PVA-1; 24 h, PCL-g-PVA-2; and 48 h, PCL-g-PVA-3

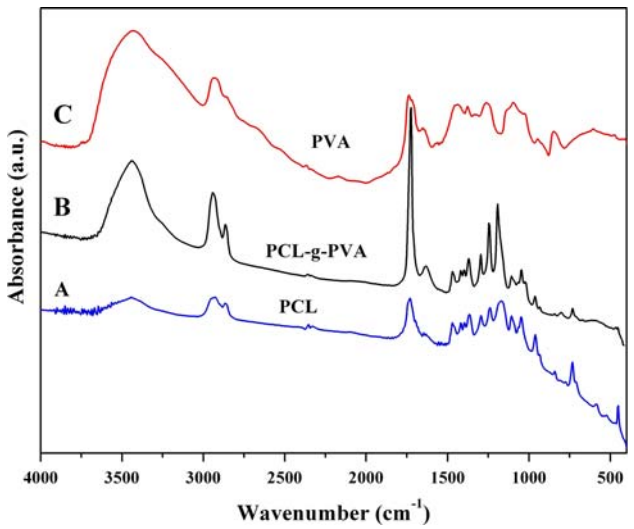



**Fig. 6** FT-IR spectra of pure PCL (A), PCL-g-PVA co-polymer (B) and pure PVA (C)

absorption band at 1639 cm$^{-1}$ was observed in the grafted copolymer which accounts for asymmetric stretching vibrations of carbonyl group of caprolactone in the PCL side chains. The peak at 720 cm$^{-1}$ represents $CH_2$ bending of caprolactone chains. These results simultaneously support formation of PCL polymer and grafting PCL on PVA.

### 3.2 Thermal Properties of PCL-g-PVA copolymer

Figure 7a represents the thermal analysis data for pure PVA and PCL-g-PVA samples. Figure 7b reveals plotting of the first derivatives to extract more accurate information. As shown in Fig. 7b, PVA first derivative curve has two peaks at around 336 and 442°C, we think appearance of these two peaks denote to the variety in the molecular weights of the used polymer. In other words, the first derivative curve of pure PVA detects that the used polymer does have many macromolecules with different molecular weights. Since TGA analysis has been conducted in nitrogen atmosphere, so, the first peak can be explained as decomposition of the low molecular weights molecules; however, the next peak represents the higher molecular weights ones. Grafting of PVA by PCL affects the thermal properties. As shown in Fig. 7b, PCL-g-PVA does have two peaks also but at different temperatures; 305 and 436°C. Compared with the two obtained peaks in case of pure PVA one can say that grafting PVA by PCL slightly decreases the decomposition temperatures. Appearance of two peaks in case of the grafted polymer supports the hypothesis that the used PVA has wide molecular weight range, so, the obtained two peaks in this plot can be investigated as the grafted lower and higher molecular weights of PVA macromolecules. It is noteworthy mentioned that the obtained thermal analysis results provide another proof that the final product is not physically blended mixture but PCL has been chemically bonded with PVA.

### 3.3 Nanoparticles formation

Generally, block and graft copolymers having amphiphilic nature containing water and oil loving segments use to self-assemble in aqueous solution which leads to form micelle

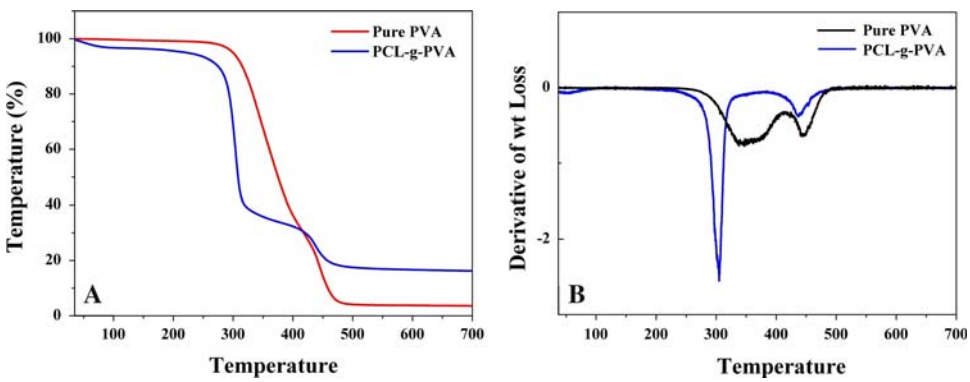



**Fig. 7** Thermal behavior of pure PCL and PCL-g-PVA (**a**), derivative of weight loss from pure PCL and PCL-g-PVA (**b**)

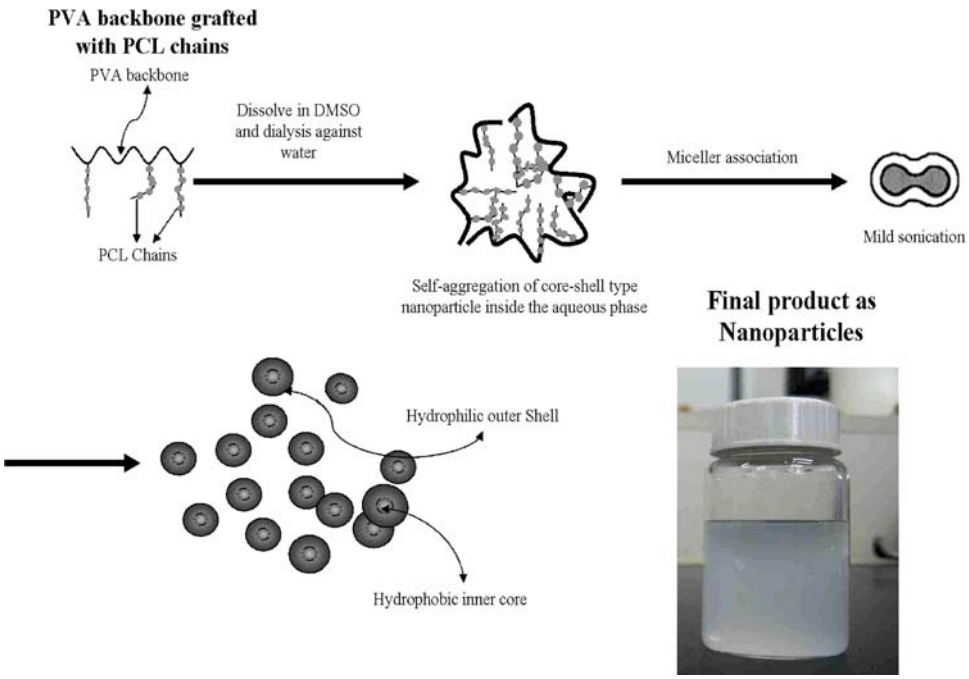
[15]. This phenomenon can be exploited in our case since we have synthesized copolymer being amphiphilic in nature due to possessing PCL as hydrophobic chains grafting hydrophilic PVA backbone. For converting the obtained copolymer in the desired shape of NPs, we should know the solubility parameters of synthesised polymer. For this reason, we tried to dissolve the grafted polymer in various solvents; such as acetonitrile, dichloromethane, chloroform, acetone, toluene, THF, DMF and DMSO in order to examine its solubility characteristics (data not shown). Among these various solvents, we found DMSO was the only satisfactory solvent for this kind of grafted polymer. It is an admitted fact that a self-assembly of block copolymers can be fulfilled by different techniques, like direct dissolution, emulsion/solvent evaporation and solvent-diffusion/dialysis [16–18]. However in our case we followed the direct dialysis technique because of the high boiling point of DMSO which arise difficulty to remove the solvent. Therefore, techniques like direct dissolution, solvent evaporation/film formation and co-solvent evaporation methods will not be applicable with this type of polymer solution to form NPs.

As aforementioned in Sect. 3.1 (NMR results), PCL DP and content in the grafted copolymer obtained increases with increasing the reaction time (Table 1). Incorporating of high amount of oil loving PCL part in the final product misbalances the amphiphilic nature of final grafted copolymer NPs stable for long time. Consequently, with increasing the hydrophobic content we found unstable micelles due to high amount of hydrophobic PCL. Therefore, the product with DP of 2.1 (as indicated in Table 1) was taken as a material of interest for the further study. The micelles formed by this product were stable for more than 1 month other than formed from copolymer batches with DP of 3.5 and 4.2, respectively, as observed by constant DLS analyses and solution transparency test.

Scheme 1 shows a schematic illustration of dialysis process which results in self-aggregation of core-shell type NPs inside the aqueous phase. As shown in this illustration, the prepared polymer as it is expected has PVA as backbone and PCL as chains, originating from PVA. Due to gradual water exposure, PCL gets warped up the PVA chain in a way putting it away from the water molecules, hence, the NPs shape turned to the second stage shown in the scheme. With the passage of time during dialysis process, more water molecules enter the dialysis tube and force the PCL chains to more hiding and condensing. Later on, NPs solution was subjected to sonic vibration in the third stage.

### 3.4 Particle size and distribution

Particle size and distribution is an important key point for NPs to be used as carrier of drugs as it affects drug release kinetics from NPs. Generally, particles with size in the range of 10 to 1000 nm, which can be either spherical or vesicular are categorized as NPs [19]. Similarly, NPs having size less than 5 μm would be taken up via the lymphatics, however, NPs less than 500 nm can across the membrane of epithelial cells through endocytosis [20, 21].




**Scheme 1** Schematic illustration of core-shell type PCL-g-PVA NPs

**Table 2** DLS and ELS results for the as prepared and drug loaded NPs

| Property of samples | PCL-g-PVA NPs before drug loading | PCL-g-PVA NPs loaded with *paclitaxel* | PCL-g-PVA NPs loaded with *doxorubicin* |
|---|---|---|---|
| Average particle size (nm) | 361 | 457 | 981 |
| Zeta potential (mV) | −218 | −183 | −147 |

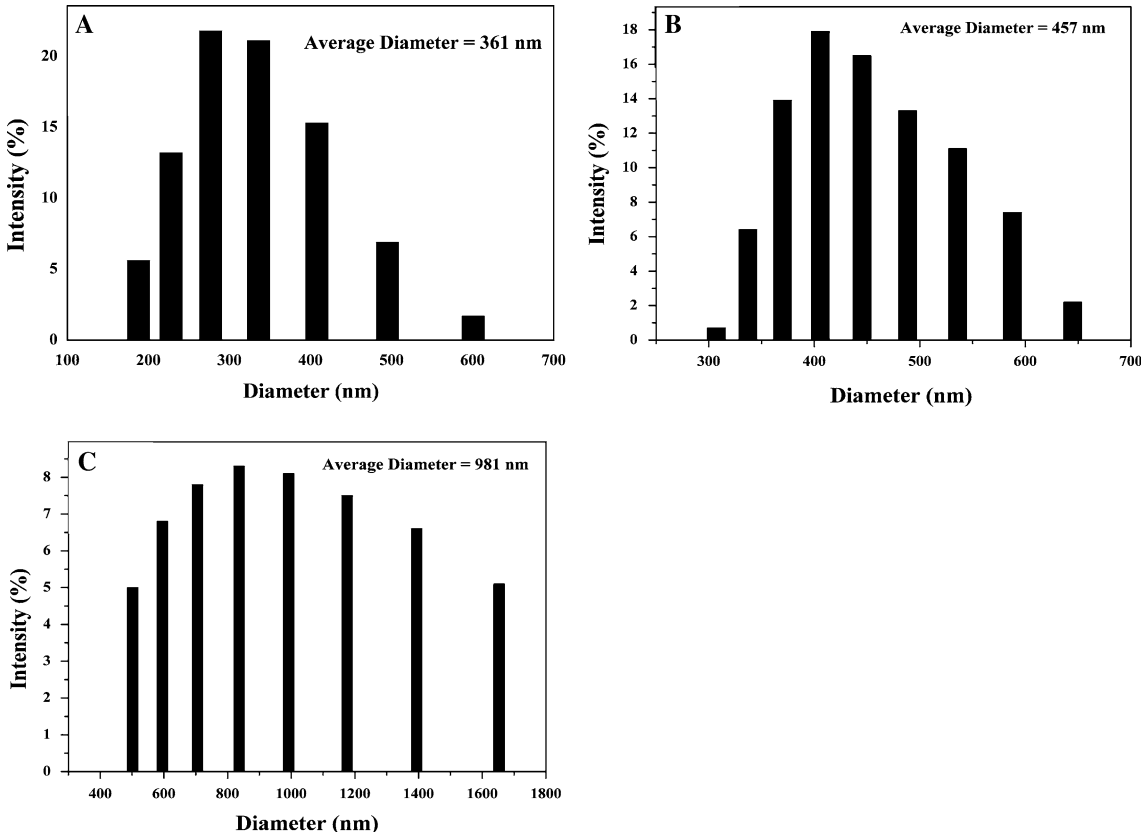



**Fig. 8** Hydrodynamic size measurements of as prepared PCL-g-PVA NPs (**a**), hydrodynamic size measurements PCL-g-PVA NPs loaded with paclitaxel (**b**) and of PCL-g-PVA NPs loaded with doxorubicin (**c**)

Generally, efficient NPs for delivering the drug molecules should have small size to have more surface area to volume ratio for expressing more drugs in required sites in human body. At this stage, Table 2 and Fig. 8 show the hydrodynamic size measurements by DLS of the as-prepared and drug-loaded NPs. Results indicated average mean hydrodynamic diameter of the as-prepared NPs was almost 361 nm. However, when the NPs were loaded with different drugs; an increase in the size was observed. As shown in Table 2, in a case of *paclitaxel*, the size increased to 457 nm, while NPs loaded by *doxorubicin* do have size of 981 nm. Increase in hydrodynamic diameters in case of both drug loaded formulation can be denoted to incorporation and binding of drug to NPs. In both drug-loaded formulations, the NPs were well separated, unagglomerated with uni-modal size distribution.

### 3.5 Surface charge

The surface charge is an important physico-chemical factor playing role with NPs [22], $\zeta$ potential is the common indicator. Negative $\zeta$ potential value of NPs is important parameter to be used as a vehicle in drug delivery systems. High negative $\zeta$ potential is used also as an indicator for the stability of the colloids. In other words, for high negative $\zeta$ potential colloid; the NPs will be well-suspended and less likely to aggregate or form flocculation or coagulation [20]. Consequently, high negative $\zeta$ potential particles will be more easily to be taken up by the living cells due to absence of clusters due to agglomeration or coagulation. Table 2 shows $\zeta$ potential values of the prepared NPs and the loaded NPs by the utilized drugs. As shown in this table, $\zeta$ potential of the prepared NPs is highly negative;

−218 mV which indicates very good stability of the obtained colloid. Also, one can not expect formation of any NPs clusters due to agglomeration or coagulation. While in case of *paclitaxel* and *doxorubicin* loaded NPs, ζ potential were −183 and −147 mV, respectively. The reason for decrease ζ potential comes from enlargement of NPs size after drug loading as shown in the DLS results (Fig. 8). Basically, increase in particle size will decrease surface to volume ratio which consequently decreases the charges density on the NPs surface. However, the obtained ζ potentials for the loaded NPs are still in the safe range which guarantees good stability and lack of tendency of agglomeration or coagulation. These results embolden utilizing these NPs in drug delivery process.

### 3.6 Stability of the nanoparticles' suspension

Keeping in view the physiological condition of human body, the stability and degradation behavior of the NPs in aqueous environment are important parameters to mimic the designed strategy for in vivo practices. These technically important characteristic features were estimated by incubating NP suspension at 37°C in PBS with vigorous stirring for a period of 8 days; along with, samples were analyzed for hydrodynamic diameter. The obtained results are demonstrated in Fig. 9. As shown in the figure, the hydrodynamic size of the NPs expressed more values or keeping same diameter during the 8 days experimental time, this result exactly matches a previous published report about the PCL degradation behavior [23]. Overall, prepared NPs do have good particle stability to be permissible for using these NPs as drug carriers. Moreover, this result draws our attention to that if the prepared NPs were loaded by drugs; the degradation will not have effect on the drug release kinetics especially during the period of first few days which is considered the critical time in drug delivery process.

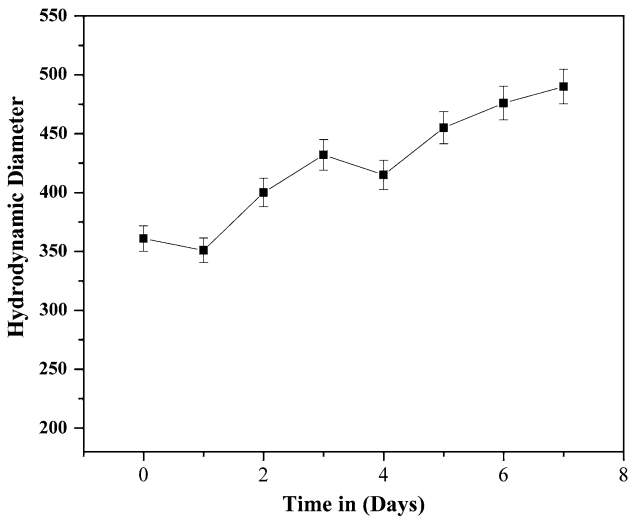



**Fig. 9** Hydrolytic stability of NPs in PBS incubated at 37°C for period of 8 days

### 3.7 Particle size and morphology

For the application of polymeric NPs in various biotechnological fields more straightly; particle size, stability and surface characteristics are the most important factors [24, 25]. In this regard, Fig. 10 shows the morphologies of PCL-g-PVA NPs analysed by BIO-TEM. As can be observed from these images, the as-prepared NPs are spherical, well disperse and uniform in distribution. Moreover, from these images one can visibly locate the PVA shell enveloping PCL core. The obtained average size of these NPs was in the range of 200 nm which is in a good agreement with the size obtained from DLS results. It is noteworthy mentioning that the hydrodynamic diameters of particles were around 361 nm, this large size in case of hydrodynamic diameter is due to swelling nature of amphiphilic polymer in aqueous solution. However, in case of analyzing NPs by TEM the NPs retain to their original diameter in dry state.

### 3.8 Drug release study

Dialysis method is a trustful way to estimate the release of drugs from NPs. The utilized membranes are semi-permeable consisting of a spongy matrix of crosslinked polymers; the pore size rating is referred to as Molecular Weight Cut Off (MWCO), which is an indirect measure of the retention performance. As such it is expected

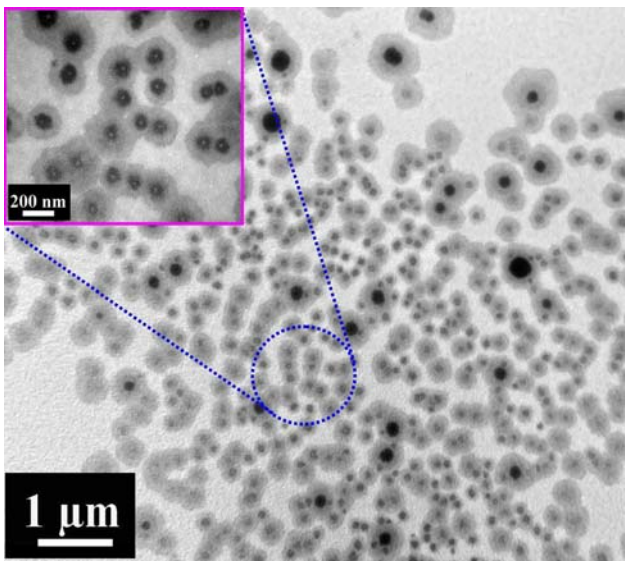



**Fig. 10** BIO-TEM images of PCL-g-PVA NPs: the insets show high magnification

considering molecular weight of the main raw material used (i.e. PVA) in preparation of our NPs, its molecular weight (MW = 65,000) is quit big enough to be retained by 12000-14000 MWCO of utilized dialysis tubing. Accordingly, exploiting such kind of dialysis tube is trustable, since these tubes will permit only the drug to pass and imprison the NPs. In order to check the ability of the synthesized NPs as drug carriers, drug release experiments were conducted as explained in the experimental section (see Sect. 2.2.4). Figure 11 shows the release results for the utilized two drugs. As shown in this figure, an initial burst release was observed with both drug types. However, with time passing; each drug released with different behavior. As shown in the figure, continuous and sustained releases have taken place with *paclitaxel* and *doxorubicin*, respectively. The reason for initial burst release for both the formulations can be accounted due to drug attached on the surfaces of NPs, so, this burst release was seen. While, in later phase for *paclitaxel*; the release from the NPs became continuous for a period of 20 days. The reason for continuous release might be due to presence of free hydroxyl groups in PCL-g-PVA copolymer which were detected by FT-IR analysis. Actually, free OH groups allow hydration and pore formation [26] that may be possible cause to show continuous release for more than 20 days. While in case of *doxorubicin*, after initial 24 h in its later phase, it was seen sustained release for a period of 15 days. The reason for difference in release pattern of two drug formulations can be retained to the location of the drug in the NPs. In case of NPs loaded with paclitaxel, the drug was imprisoned inside the core of NPs which makes less contact with outer media; consequently, continuous release for such long time is acceptable. However, in case of *doxorubicin*, the drug was physically attached with outer hydrophilic PVA shell surface; due have continuous contact with outer media and thereby allows release of drug at study rate so, sustained release of drug was obvious. Also, drug solubility might have an effect; *doxorubicin* is more soluble in water. It is noteworthy to point out that the surfaces of the obtained NPs are strongly rough and not smooth ones, so, there are many sites to entrap the *doxorubicin* drug, that can be satisfactory explanation for the long time sustain drug release for 15 days. Therefore, although the *doxorubicin* drug is relatively more soluble in water and existing in the surface of the particles but due to well attachment and surface roughness it reveals slow release. Meanwhile, after the controlled release; the remaining drug in the dialysis bags for both drug formulations were spectrophotometrically estimated and found to be from 10 to 15%. Overall, the produced NPs formulations could provide a low therapeutic index and improved patient compliance.

In order to get full confirmation that really the prepared NPs are playing important role in drug release and

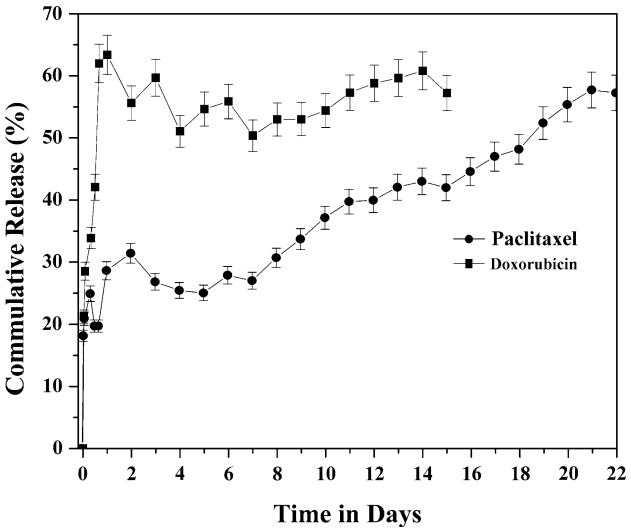



**Fig. 11** In vitro drug release profiles of paclitaxel and doxorubicin loaded NPs and in phosphate buffer, pH 7.4, at 37°C

simultaneously study the possibility of binding/absorbance of drugs with dialysis membrane, drug release experiments of the both pure drugs have been conducted by the same procedure without exploiting the NPs. In this regard, Fig. 12 shows the release results obtained in a case of placing only pure drug inside the dialysis tube. As shown in this figure, the whole drug was released from dialysis within 1–2 h. Moreover, the final drug concentration in the PBS was higher than the case of exploiting the NPs, this indicates that the prepared NPs do contain certain amount of drug. This comparison clarifies that NPs do possess the drugs which get release in such long times. This satisfactorily indicates the advantage of using PCL-g-PVA NPs in future drug delivery system. Meanwhile, by full release of free drugs from dialysis tubing one can clearly say that

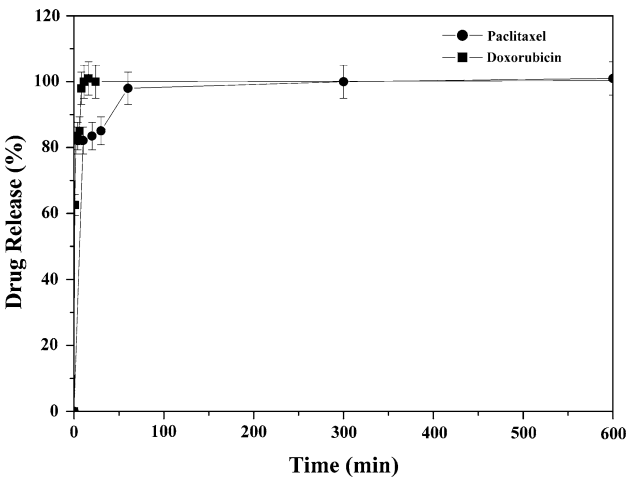



**Fig. 12** In vitro drug release profiles of pure paclitaxel and doxorubicin in phosphate buffer, pH 7.4, at 37°C

there is no binding/adsorbance of drug with dialysis membrane which may influence the release kinetics from NPs.

It is noteworthy to point to that the drug percentages demonstrated in the drug release figure for both drugs have been estimated as a percentage of the original amount of drug used in the loading process and not based on the successfully loaded amount. Actually, we have utilized this strategy because we think it is more significant since the unloaded drug is considered as waste, so, we aimed to show how much drug can be exactly exploited by the proposed NPs. It is economically feasible methodology.

## 4 Conclusions

ε-caprolactone and PVA can be used to synthesize a grafted copolymer. $^{1}$H NMR and FT-IR analysis has confirmed side chain polymerization of the used caprolactone monomer as well as attached the formed PCL with the PVA chain to produce PCL-g-PVA. Moreover, thermal gravimetric analysis has confirmed the grafting process. The obtained copolymer was able to self-aggregate into NPs by direct dialysis method. The prepared NPs have high negative ζ potential which makes them adequate to be used as drug carriers. Micelles remained stable for a period of 8 days in PBS. *Paclitaxel* and *doxorubicin* drugs were released in sufficient times from the prepared nanoparticles; while in a case of pure drugs, 1 to 2 h was enough to totally release the drugs. The physiochemical characterizations and the drug release results strongly recommended use of the prepared particles in drug delivery systems for both hydrophobic and hydrophilic drugs.

**Acknowledgement** This work was supported by Korean Research Foundation Grant founded by Korean Government (MOEHRD) (The Center for Health Care Technology, Chonbuk National University, Jeonju 561–756 Republic of Korea).

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