Development of Nonsurfactant Cyclodextrin Nanoparticles Loaded With Anticancer Drug Paclitaxel

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ABSTRACT: In the current formulation of clinical use paclitaxel (PCX) is associated with solubilizers that may produce severe side effects. In this study, PCX was complexed to an amphiphilic cyclodextrin (CD), 6-O-CAPRO-β-CD, capable of forming nanoparticles spontaneously in order to mask its physicochemical properties via the formation of inclusion complexes of the drug with amphiphilic CD before the nanoparticle is formed. Complexes have been characterized with various techniques such as 1H NMR, Fourier Transform Infrared (FTIR), differential scanning calorimetry (DSC) and scanning electron microscopy (SEM) confirming the formation of inclusion complex between PCX and 6-O-CAPRO-β-CD. Nanospheres and nanocapsules were prepared directly from the preformed PCX/6-O-CAPRO-β-CD inclusion complex by the nanoprecipitation technique, showing a size from 150 to 250 nm for nanospheres and from 500 to 500 nm for nanocapsules. Zeta potentials of the nanospheres and nanocapsules indicate stable colloidal dispersions within the range of −18 to −39 mV. A 12-month physical stability was demonstrated for blank nanoparticles. PCX encapsulation was high with three-fold increase in loading when nanoparticles are prepared directly from preformed inclusion complexes of the drug with 6-O-CAPRO-β-CD. In vitro liberation profiles of PCX from CD nanoparticles show a prolonged release profile for this drug up to 12 h for nanospheres and 24 h for nanocapsules. © 2007 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 97:1519–1529, 2008

Keywords: cyclodextrins; nanospheres; nanocapsules; complexation; NMR; thermal analysis; controlled release; physical stability; FTIR; particle sizing

INTRODUCTION

Paclitaxel (PCX) is an antitumor agent with a unique mechanism of action promoting the assembly of microtubules from tubule dimers and prevents them from depolarizing.1–3 This leads to the loss of normal microtubule dynamics necessary for cell division and other vital processes and consequently causes cell death. The ability of this drug to stabilize microtubules makes it significantly effective against various types of solid tumors including breast cancer, advanced ovarian carcinoma, lung cancer, head and neck carcinomas, and acute leukemias.4,5 However, the clinical application and the bioavailability of PCX is mainly limited by its narrow therapeutic index and very poor solubility in water (≤0.5 mg/L) and other pharmaceutically acceptable solvents. The current formulation of injectable PCX (Taxol®) consists of Cremophor EL

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and dehydrated alcohol at 50:50 v/v ratio. This vehicle has been demonstrated to cause severe hypersensitivity reactions upon administration and has shown incompatibility with common PVC intravenous infusion sets.\textsuperscript{6–10} To eliminate Cremophor EL from PCX injectable formulations, alternative delivery systems have been suggested including parenteral emulsions,\textsuperscript{11,12} liposomes,\textsuperscript{13,14} nanoparticles of different polymeric structures,\textsuperscript{15–20} water-soluble drugs and conjugates,\textsuperscript{21–23} and inclusion complexes with CDs.\textsuperscript{24–28}

Recently Abraxane\textsuperscript{1}, a Cremophor-free, albumin-bound nanoparticle PCX of mean diameter of 130 nm has been approved by the FDA for the therapy of recurrent metastatic breast cancer that does not respond to combination therapy. Albumin has been used to formulate PCX in nanoparticulate form for cancer therapy.\textsuperscript{29,30} In fact, administration of poorly soluble anticancer drugs in nanoparticulate carrier systems seems to be advantageous due to reduced size of these delivery systems (<400 nm) which help escape from reticuloendothelial system RES uptake and facilitate the extravasation through the leaky vasculature typical of the tumor site as well as the avoidance of Cremophor as the solubilizer. This is believed to reduce side effects and increase therapeutic efficacy while altering the tissue distribution and the pharmacokinetics of the anticancer drug.\textsuperscript{31,32} Furthermore it has been demonstrated that nanoparticles may overcome MDR type P-gp increasing the drug content inside the neoplastic cells.\textsuperscript{33} This may also help increase the therapeutic efficacy of PCX since an acquired resistance to the drug has already been reported.\textsuperscript{34}

Amphiphilic cyclodextrins (CDs) are obtained by the chemical per-modification of natural CDs (\(\beta\)-CD or \(\gamma\)-CD) by the selective substitution of aliphatic chains of varying length (2C to 18C), structure (linear or branched) linked with varying bonds (ester, ether, amide, thio, fluoro) of high purity.\textsuperscript{35–37} These CD derivatives were demonstrated to yield nanospheres or nanocapsules spontaneously using the nanoprecipitation technique with or without the presence of surfactants.\textsuperscript{36} Some amphiphilic \(\beta\)-CD derivatives such as \(\beta\)-CDC6 modified on the secondary face with 6C aliphatic esters and 6-N-CAPRO-\(\beta\)-CD modified on the primary face with a 6C aliphatic amide were demonstrated to give stable nanoparticles of high drug loading capacity and reduction of burst effect during the drug release process when nanoparticles are prepared directly from preformed drug/amphiphilic CD inclusion complex.\textsuperscript{38–40}

The objective of this study was to combine for the first time two different alternative approaches to PCX formulation which are complexation to CDs and incorporation of PCX into nanoparticulate carrier systems. For this reason, a new amphiphilic CD derivative, 6-O-CAPRO-\(\beta\)-CD, modified on the primary face with 6C aliphatic esters suggesting biodegradability, was synthesized to form inclusion complexes with PCX. Nanoparticles were prepared directly from the preformed inclusion complex in order to enhance the therapeutic concentration of the drug while avoiding the use of Cremophor, to improve the physical stability of PCX, to achieve high encapsulation efficiency, and controlled release profile.

**EXPERIMENTAL SECTION**

**Materials**

Reagents and chemicals were used as received in the synthesis of 6-O-CAPRO-\(\beta\)-CD. \(\beta\)-CD 100% pure according to nuclear magnetic resonance spectroscopy was extensively dried under vacuum over phosphorous pentoxide before use. PCX seen in Figure 1a was a kind gift of Zhejiang Pharmaceuticals, Hangzhou, China (EA648838320CN). Cremophor\textsuperscript{1} EL used to solubilize PCX was purchased from BioChemika/Fluka, Buchs, Belgium. Miglyol 812 used as the oil phase in nanocapsule preparation was purchased from Condea Chimie, Witten, Germany. All other reagents were of HPLC grade and were used without further purification. Ultrapure water MilliQ (Millipore Simplicity 185, Molsheim, France) was used in the preparation of nanoparticles.

**Synthesis and Analytical Characterization of 6-O-CAPRO-\(\beta\)-CD**

Amphiphilic CD derivative, 6-O-CAPRO-\(\beta\)-CD (Fig. 1b) was synthesized according to the previously described technique.\textsuperscript{35} Briefly, 6-O-CAPRO-\(\beta\)-CD was obtained by iodination of the primary hydroxyl groups and reaction with cesium hexanoate which proved to be a good nucleophile\textsuperscript{35} in DMF resulting in the per-modification of primary hydroxyls with 6C aliphatic esters. Final product of 1.9 g (92%) white powder was characterized by
elemental analysis, $^1$H NMR spectrometry, Fourier Transform Infrared (FTIR) spectroscopy, differential scanning calorimetry (DSC) analysis, and melting point determination to confirm the product’s purity, thermal characteristics, and structure to be in accordance with the previously obtained results.\textsuperscript{35}

**Preparation of PCX/6-O-CAPRO-\(\beta\)-CD Inclusion Complexes**

Colyophilization technique was used to prepare inclusion complexes of the drug with amphiphilic CD derivative 6-O-CAPRO-\(\beta\)-CD. 1:1 and 1:2 molar ratios of drug: CD were studied. In this technique, PCX and 6-O-CAPRO-\(\beta\)-CD were dissolved in 20 mL of absolute alcohol in fixed amounts and this solution was poured into 40 mL of distilled water in a round-bottomed flask. The suspension was stirred for equilibration at room temperature for 7 days. Organic solvent was then evaporated under vacuum and the resulting suspension was lyophilized to obtain the inclusion complex in dry powder form.

**Characterization of PCX/6-O-CAPRO-\(\beta\)-CD Inclusion Complexes**

DSC was used to determine the thermal behavior of PCX, 6-O-CAPRO-\(\beta\)-CD, and the 1:1 and 1:2 M complexes. DSC analysis was performed with a Dupont DSC912 (Wilmington, DE) instrument within the temperature range of 25-250°C under nitrogen atmosphere. Samples weighing approximately 3 mg were heated in hermetically sealed aluminum pan at a rate of 10°C/min. Fourier Transform Infrared (FTIR) Spectroscopy was performed with a Nicolet 520 FTIR spectrophotometer (Waltham, MA), using discs of each sample and previously prepared KBr containing 0.01 g of sample and 0.1 g KBr between wavelengths 40 and 4000 cm\(^{-1}\). $^1$H NMR spectra at 400 MHz were taken in CDCl\(_3\) and DMSO with a Varian Mercury 400 MHz. Chemical shifts are given to external tetramethylsilane at 0 ppm with calibration using solvent signals. Scanning electron microscopy (SEM) was used to determine the crystalline structure of PCX, 6-O-CAPRO-\(\beta\)-CD, and their complexes with a JEOL SEM ASID-10 instrument, Tokyo, Japan. Samples were fixed on metal plates and sputtered with gold–palladium mixture at a thickness of 100 A˚ and observed at an accelerated voltage of 80 kV.

**Preparation of Blank and PCX-Loaded Nanoparticles**

Nanospheres were prepared according to the nanoprecipitation technique introduced by Fessi et al.\textsuperscript{41} According to this technique, an organic phase consisting of 6-O-CAPRO-\(\beta\)-CD (1 mg) dissolved in absolute alcohol (1 mL) was added with an Eppendorf injector at room temperature under constant stirring to an aqueous phase consisting of only Ultrapure Water (MilliQ).
Nanospheres formed spontaneously. This dispersion was stirred for equilibrium for 30 min followed by evaporation of organic solvent under vacuum to the desired volume (2 mL). To obtain nanocapsules, the same technique is used with the exception of an oil phase of Miglyol 812 (50 μL) added to the organic phase.

Drug-loaded nanoparticles were prepared with either the conventional loading (CL) technique and the previously reported high-loading technique (HL) based on the preparation of nanoparticles from preformed inclusion complexes.\textsuperscript{38,39} The loading techniques can be described briefly as follows:

- **Conventional loading (CL):** Nanoparticles are loaded by adding fixed amount of ethanolic PCX solution (200 μg) to the organic phase during preparation.

- **Preloading technique (PL):** Nanoparticles are prepared by dissolving only a fixed amount (1 mg) of PCX/6-O-CAPRO-β-CD complex in the organic phase for the preparation.

- **High-loading technique (HL):** Nanoparticles are prepared from fixed amount of PCX/6-O-CAPRO-β-CD complex (1 mg) with an overloading of ethanolic PCX solution (200 μg) in the organic phase during preparation.

### Characterization of Blank and PCX-Loaded Nanoparticles

Particle size distributions (mean diameter (nm), polydispersity index) of nanoparticles were determined by quasi-elastic light scattering using Malvern Zetasizer, Malvern Instruments, Worcestershire, UK. Measurements were realized in triplicate at a 90° angle at 25°C. Zeta potential values were taken using the Malvern Zetasizer, Malvern Instruments, also in triplicate at 120° angle and 25°C with suitable dilution of samples with deionized water. Atomic force microscopy (AFM) was used to image the PCX-loaded nanoparticle dispersions with a Q-Scope\textsuperscript{350} Multimode Atomic force Microscope, Ambios & Quasant, Santa Cruz, CA.

Physical stability of blank CD nanoparticles was determined through a 12-month follow-up period during which the samples were stored as aqueous nanoparticle dispersions at 8°C. Repeated measurements of particle size and zeta potential were performed in order to evaluate the long-term physical stability of the nanoparticles. Entrapment efficiency of PCX to amphiphilic CD nanoparticles was determined by the following technique; free undissolved drug in the nanoparticle dispersion was centrifuged at 5000 rpm and the resulting supernatant was separated and lyophilized (HETO Lyolab Freeze Dryer, Allerod, Denmark). The dry powder consisting of nanoparticles and nanoparticle-bound PCX was then dissolved in acetonitrile: water (70:30 v/v) and PCX quantity was assayed with an HPLC technique using HP 1100 Agilent Series instrument with a mobile phase of acetonitrile:water (70:30), flow rate of 1 mL/min, injection volume of 50 μL at 227 nm with a diode array detector using a reverse phase column C-18 octadecylsilane (ODS) (Hichrom, Berkshire 5 Cl8 column, 250 × 4.6 mm, UK) giving a retention time of 4.2 min for PCX. The HPLC technique was analytically validated (linearity $r^2$: 0.9999, repeatability CV%: 0.4%, reproducibility CV%: 1.1%, LOD: 0.028 μg/mL, LOQ: 0.095 μg/mL).

Drug loading data were expressed in terms of entrapped drug quantity, entrapment efficiency, and associated drug %. Entrapped drug quantity is the drug quantity determined in nanoparticle sample (μg/mL). Entrapment efficiency and associated drug % values were calculated according to the following formula:

\[
\text{Entrapment efficiency} = \frac{\text{Entrapped drug quantity (μmol)}}{\text{Initial CD amount (μmol)}} \times 100
\]

\[
\text{Associated drug %} = \frac{\text{Entrapped drug quantity (μg)}}{\text{Initial drug amount (μg)}} \times 100
\]

In vitro PCX release profiles from 6-O-CAPRO-β-CD nanospheres and nanocapsules were determined in capped Eppendorf tubes in a 20 mL of medium of isotonic phosphate buffer saline pH 7.4 containing 0.1% polysorbate 80 for sink conditions at 37°C in a thermostated shaker bath (Memmert, Schwabach, Germany) at 80 rpm. At given time intervals 0.5 mL of sample was taken from the release medium and replaced with fresh medium at 37°C. Samples were then centrifuged at 13500 rpm for 15 min to precipitate the nanoparticles and the clear supernatant was freeze dried. Resulting dry powder was dissolved in 0.5 mL of mobile phase and assayed for PCX content.
RESULTS AND DISCUSSION

Characterization of 6-O-CAPRO-β-CD

6-O-CAPRO-β-CD, amphiphilic CD derivative modified on the primary face with 6C aliphatic ester was characterized after synthesis by multiple techniques including 1H NMR spectroscopy, FTIR spectroscopy, DSC, and elemental analysis. 1H NMR data for 6-O-CAPRO-β-CD are as follows: 1H NMR (δH, 400 MHz, DMSO-d6): 7.99 (DMF, H–C=O), 6.04 (dd, –OH), 5.86 (d, –OH), 4.92 (d, H-1), 4.32 (d, -H-6), 4.21 (dd, H-6), 3.86 (t, H-5), 3.66 (m, H-3), 3.37 (m, H-4), 3.4 (DMSO-H2O), 2.7, 2.9 (DMF, -N-(CH3)2), 2.54 (DMSO), 2.31 (m, CH2–CO–), 1.52 (m, CH2–CH2–CO–), 1.28 (m, –(CH3)2), 0.88 (t, CH3).

FTIR spectrum (data not shown) of 6-O-CAPRO-β-CD gave the following typical bands confirming the ester structure of this derivative: O–H stretching vibrations at 3700–3100 cm–1, aliphatic C–H stretching vibrations at 3000–2850 cm–1, C=O stretching bands characteristic of the ester group at 1800–1700 cm–1.

DSC thermogram taken between 25 and 250°C shows a distinct and sharp melting endotherm for 6-O-CAPRO-β-CD at 229°C (data not shown). Table 1 shows the elemental analysis data for the newly synthesized 6-O-CAPRO-β-CD proving the purity of the final product.

Characterization of PCX/6-O-CAPRO-β-CD Inclusion Complexes

The interaction at the solid state and in solution between PCX and CD cavity was evaluated by different techniques such as DSC analysis, FTIR spectroscopy, 1H NMR spectrometry, SEM imaging.

Figure 2 displays the DSC thermograms of lyophilized PCX, lyophilized 6-O-CAPRO-β-CD, 1:1 and 1:2 molar ratio complexes of the drug, and CD. As seen from the DSC thermograms, melting endotherm of 6-O-CAPRO-β-CD at 229°C and melting and decomposition endotherms and dehydration at 100°C of PCX at 224 and 232°C have disappeared or broadened suggesting the absence of free crystalline drug within the colyophilizate. FTIR spectra for PCX, 6-O-CAPRO-β-CD, and their 1:1 and 1:2 complexes are seen in Figure 3 indicating a reduction in the O–H stretching vibration band at 3300 cm–1. The peaks about 1500 and 1200 cm–1 relative to aromatic ring and C–O–C stretching respectively show significant changes. The peak about 1700 cm–1 corresponding to aryl and saturated ketone groups also indicates a possible interaction through the aromatic rings of the drug molecule seen in Figure 1a. These data indicate that there exists a chemical interaction between PCX and 6-O-CAPRO-β-CD which is most probably indicative of a partial inclusion of the drug within the amphiphilic CD cavity. This interaction is more pronounced for the 1:2 molar ratio complex as seen in Figure 3.

1H NMR spectra of PCX, 6-O-CAPRO-β-CD, and 1:1 and 1.2 molar ratio complexes were taken at 400 MHz and shifts in the signals of H-3 and H-5 along with other protons of amphiphilic CD were evaluated in terms of a possible inclusion in CD cavity which is expected to cause negative shifts in the internal protons (H-3 and H-5) of the CD cavity. Table 2 represents the chemical shift values of 6-O-CAPRO-β-CD protons before and after complexation to PCX. It can be observed that internal protons H-3 and H-5 display shifts of 0.02 to 0.03 ppm for 1:1 and 1.2 molar ratio complexes. More interestingly, H-6 and H-6’ which are the protons of the aliphatic ester grafted to the primary hydroxyl neighboring the CD glucopyranose unit has also shifted significantly as seen from Table 2. This suggests that PCX molecule has entered the CD cavity and also interacted with the aliphatic chains grafted to the primary face. Thompson et al. has proposed that leaving the secondary face which is the wide side of the cavity unsubstituted may facilitate the entrance of hydrophobic drug molecule to the cavity. Modification of the primary face with long aliphatic chains contributes to the complexation ability of the CD derivative by providing a second hydrophobic zone to interact with after the CD cavity.

Table 1. Elemental Analysis Data of Amphiphilic CD, 6-O-CAPRO-β-CD

<table>
<thead>
<tr>
<th>Amphiphilic CD</th>
<th>Empirical Formula</th>
<th>Molecular Weight (g/mol)</th>
<th>Theoretical Percentage</th>
<th>Practical Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-O-CAPRO-β-CD</td>
<td>C84H140O42</td>
<td>1820</td>
<td>(C%) 55.3</td>
<td>(C%) 50.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(H%) 7.7</td>
<td>(H%) 7.3</td>
</tr>
</tbody>
</table>
Figure 2. DSC thermograms of PCX, 6-O-CAPRO-β-CD, 1:1 and 1:2 molar ratio complexes of PCX/6-O-CAPRO-β-CD.

Table 2. Chemical Shift Values (ppm) of 6-O-CAPRO-β-CD Protons before and after Complexation to PCX in 1:1 and 1:2 Molar Ratio

<table>
<thead>
<tr>
<th>Protons</th>
<th>( \delta_{CD} ) (^a)</th>
<th>( \delta_{1:1} ) (^b)</th>
<th>( \delta_{1:2} ) (^b)</th>
<th>( \Delta \delta_{1:1} ) (^c)</th>
<th>( \Delta \delta_{1:2} ) (^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-1</td>
<td>4.92</td>
<td>(—) (^e)</td>
<td>(—) (^e)</td>
<td>(—) (^e)</td>
<td>(—) (^e)</td>
</tr>
<tr>
<td>H-3</td>
<td>3.66</td>
<td>3.64</td>
<td>3.64</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>H-4</td>
<td>3.37</td>
<td>3.36</td>
<td>3.37</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>H-5</td>
<td>3.86</td>
<td>3.83</td>
<td>3.84</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>H-6</td>
<td>4.32</td>
<td>4.31</td>
<td>4.31</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>H-6'</td>
<td>4.21</td>
<td>4.16</td>
<td>4.16</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

\(^a\)Free state. \(^b\)Complexed state. \(^c\)\(\Delta \delta_{1:1} = \delta_{CD} - \delta_{1:1}\). \(^d\)\(\Delta \delta_{1:2} = \delta_{CD} - \delta_{1:2}\). \(^e\)Peaks could not be separated since H-1 proton of 6-O-CAPRO-β-CD and H-5 proton at the taxane core of PCX have given resonance at the same ppm value.

Figure 3. FTIR spectra of PCX, 6-O-CAPRO-β-CD, and 1:1 and 1:2 molar ratio complexes.
Figure 4. SEM photomicrographs of (a) PCX (b) 6-O-CAPRO-β-CD, (c) 1:1 complex, and (d) 1:2 complex.

Table 3. Particle Size and Zeta Potential Values for Blank and PCX-Loaded Amphiphilic CD Nanoparticles

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Particle Size (Mean Diameter, nm, ±SD)</th>
<th>Zeta Potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank nanosphere</td>
<td>157 ± 5</td>
<td>−13.8 ± 2</td>
</tr>
<tr>
<td>Blank nanocapsule</td>
<td>587 ± 36</td>
<td>−14.7 ± 4</td>
</tr>
<tr>
<td>CL nanosphere</td>
<td>219 ± 32</td>
<td>−19.6 ± 1</td>
</tr>
<tr>
<td>PL nanosphere</td>
<td>232 ± 5</td>
<td>−18.8 ± 2</td>
</tr>
<tr>
<td>HL nanosphere</td>
<td>263 ± 24</td>
<td>−34.6 ± 5</td>
</tr>
<tr>
<td>CL nanocapsule</td>
<td>606 ± 37</td>
<td>−32.5 ± 3</td>
</tr>
<tr>
<td>PL nanocapsule</td>
<td>450 ± 31</td>
<td>−37.1 ± 2</td>
</tr>
<tr>
<td>HL nanocapsule</td>
<td>639 ± 37</td>
<td>−33.0 ± 5</td>
</tr>
</tbody>
</table>
indicate that the needle-like crystalline structure typical of PCX does not exist in the complexes which both show an amorphous state. Figure 4a–d represents the SEM photomicrographs of the drug, CD, and the different complexes. This change in the crystalline form could be a result of the colyophilization process which is very similar to the nanoprecipitation process used to prepare the nanoparticles.44

Characterization of Blank and PCX-Loaded Nanoparticles

Particle size and zeta potential values of blank and PCX-loaded 6-O-CAPRO-β-CD nanoparticles prepared with different loading techniques are seen in Table 3. The data suggest that loaded nanoparticles are slightly larger than blank nanoparticles probably due to the adsorption of the drug on the particle surface. Nanocapsules are limited to the oil droplet size45 that form during the preparation so they are considerably larger than nanospheres but still in the appropriate nm range for injectable use. Nanospheres in particular are around 150–250 nm which suggests favorable properties for injectable systems, possibility to escape from RES uptake, and leaking from the vasculature surrounding the tumor site resulting in an eventual passive targeting of the system.46 The sizes of nanospheres were believed to be slightly affected by the preparation technique being preloading, CL or high-loading due to the different amounts of drug entrapment. Zeta potentials of the blank nanoparticles are around −19 mV with a slight increase up to −37 mV for loaded formulation. It is believed that this increase in zeta potential is due to the charge of the adsorbed PCX on the particle surface. In fact, zeta potential of PCX dispersion in buffer PBS was determined to be −30 mV confirming this theory.

Long-term physical stability of the blank nanoparticles was measured by repeated particle size and zeta potential measurements. The data are presented in Figures 5 and 6 for particle size and zeta potential measurements, respectively. The data indicate the 12-month physical stability of 6-O-CAPRO-β-CD nanospheres and nanocapsules in aqueous dispersion form upon storage at 8°C. Changes in the particle size and zeta potential during the 12-month follow-up period are not statistically significant (p < 0.05).

Drug loading values of PCX are seen in Table 4 in terms of entrapped drug quantity, entrapment efficiency, and associated drug percentage. It is seen that loading techniques increase drug loading values significantly up to threefold for high-loaded nanoparticles. It is also observed that nanocapsules entrap almost twofold PCX when compared to nanospheres. This may be a result of the affinity of this very poorly soluble drug to the oily core of the nanocapsules. It has been reported that drug physicochemical properties including octanol: water partition coefficient, k1:1 affinity constant for CD, and aqueous solubility affect loading characteristics significantly.47 In accordance with this fact, PCX with its very poor water solubility, high partition coefficient, and demonstrated affinity to CDs show a high encapsulation efficiency to CD nanoparticles.

In vitro release of PCX was assessed from nanospheres and nanocapsules into isotonic phosphate buffer pH 7.4 containing 0.1%...
polysorbate 80. Figure 7 displays the in vitro release profiles of conventionally loaded and high-loaded nanospheres and nanocapsules. It can be seen that nanocapsules liberate the drug in a considerably slower release profile. Another factor affecting release seems to be the loading technique with CL resulting in a burst effect. PCX is released in a period of 12 h with complete release achieved in 24 h for nanocapsules. PCX’s burst effect is observed in the first 15 min for conventionally loaded formulations. However, high-loaded formulations displayed biphasic release profile with complete PCX release achieved around 12 h.

Table 4. Drug Loading Values for PCX for 6-O-CAPRO-β-CD Nanoparticles Prepared With Different Loading Techniques

<table>
<thead>
<tr>
<th>Loading Technique</th>
<th>Dosage Form</th>
<th>Entrapped Drug Quantity (µg) ±SD</th>
<th>Associated Drug Percentage (%, a/a)</th>
<th>Entrapment Efficiency (%) (µmol Drug/µmol CD) × 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL</td>
<td>Nanosphere</td>
<td>67.4 ± 0.7</td>
<td>33.7</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Nanocapsule</td>
<td>120.7 ± 0.3</td>
<td>60.4</td>
<td>43</td>
</tr>
<tr>
<td>PL</td>
<td>Nanosphere</td>
<td>65.4 ± 0.6</td>
<td>32.7</td>
<td>24.6</td>
</tr>
<tr>
<td></td>
<td>Nanocapsule</td>
<td>117.2 ± 0.5</td>
<td>58.6</td>
<td>44.2</td>
</tr>
<tr>
<td>HL</td>
<td>Nanosphere</td>
<td>143.5 ± 0.9</td>
<td>36.8</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>Nanocapsule</td>
<td>186.4 ± 0.9</td>
<td>47.8</td>
<td>68</td>
</tr>
</tbody>
</table>

CONCLUSION

In the light of the data obtained in this study, it can be concluded that amphiphilic CD nanoparticles may provide a promising alternative for non-surfactant, Cremophor-free, nanoscale carrier for injectable use of PCX. Further studies should focus on the safety and efficacy of this delivery system to ensure an improvement in the therapeutic efficacy and clinical application of this potent anticancer agent.

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