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Nano Self-assemblies Based on Cholate Grafted Poly-L-lysine Enhanced the Solubility of Sterol-like Drugs

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Abstract

The physicochemical compatibility between amphiphilic polymers and hydrophobic drugs has been recognized as an important issue for improving the drug solubilisation in polymeric micelle formulations. In this work, poly-L-lysine (PLL) grafted with cholate pendants as the only hydrophobic moiety were synthesized in order to facilitate the solubilisation of sterol drugs. Results showed that micelles formed by cholate grafted PLL encapsulated significantly higher level of prednisolone and estradiol than palmitoylated PLL micelles, whereas the solubilisation capacity of non-sterol drug (griseofulvin) is inefficient for both polymers. This suggests that higher drug-polymer incorporation can be achieved by the inclusion of hydrophobic moieties with similar architecture as the drugs, i.e. “drug-like” functional groups, which will be useful for the future design of colloidal systems for the encapsulation of specific drug.

Key words hydrophobic drug solubilisation, Amphiphilic graft copolymer, Micelle, Drug-polymer interaction.

Introduction

It has been estimated that 40% of the current drug candidates in development and marketed drugs consist of water insoluble entities (Kilpatrick 2003). Poorly water-soluble drugs present a major challenge to the pharmaceutical industry, as it can hinder or even prevent the progress of the drug into clinical use (Wenlock et al. 2003). In an attempt to improve aqueous solubility of hydrophobic drugs, traditional formulations such as oil in water emulsions, co-solvents and low molecular weight surfactants have been employed (Strickley 2004). In recent years, amphiphilic polymers have attracted much attention as solubilisers for hydrophobic drugs (Kabanov et al. 2002, Kwon 2003, Gaucher et al. 2005, Rijcken et al. 2007). In the aqueous environment, amphiphilic polymers form nano-sized self-assemblies, where a hydrophobic core is created upon the aggregation of hydrophobic moieties of the polymers. The core serves as a "container" for water insoluble drugs and thus resulted in an increased solubilisation.

Basically a good polymeric solubiliser should have favourable and stronger interactions with solubilisate than the intermolecular interactions among the solubilisate molecules (Huang et al. 2008). This is especially important for those solubilisates with highly crystalline structures (Soo et al. 2002, Marsac et al. 2009). In addition to the hydrophobic interaction, the formation of ionic complexes and hydrogen bonding between the solubiliser and the solubilisate would certainly enhance the solubilisation, due to the presence of multiple polar groups commonly found in many drug molecules (Tian et al. 2007, Huang et al. 2008). Therefore the compatibility between the drug and micelle-forming polymers becomes a major concern for the design of drug solubilisers (Nagarajan 2001, Liu et al. 2004, Gaucher et al. 2005, Attwood et al. 2007, Letchford et al. 2007, Mahmud et al. 2009). One of the widely used theoretical methods, especially on amphiphilic block copolymers, is to calculate the Flory-Huggins interaction parameter (χ_{sc}) between drug and the hydrophobic block

(Nagarajan 2001, Rekatas et al. 2001, Letchford et al. 2007). Lower calculated χ_{sc} value normally indicates better drug-polymer compatibility hence higher predicted level of solubilisation (Rekatas et al. 2001, Soo et al. 2002, Letchford et al. 2007, Mahmud et al. 2009). However it has been found that this theoretical model does not work in all cases (Marsac et al. 2006). On the other hand, in recent years a few experimental works have addressed the attachment of drug molecules or functional groups with similar chemical structure of drugs onto the polymers in order to enhance the drug-polymer interaction (Kataoka et al. 2000, Lavasanifar et al. 2002, Mahmud et al. 2009). For example, Mahmud et. al conjugated doxorubicin (DOX) to the hydrophobic block of poly(ethylene oxide)-block-poly(ε-caprolactone) (PEO-b-PCL) to favour the DOX solubilisation (Mahmuda et al. 2008), while the inclusion of cholesteryl groups in the PEO-b-PCL also resulted in a higher solubilisation of cucurbitacin I, a cholesterol drug, than the parent polymer (Mahmud et al. 2009).

Compared to block copolymers, the investigation on amphiphilic graft copolymers bearing "drug-like" pendant groups for enhancing the solubilisation of poorly water-soluble drugs is rarely reported, albeit several papers have published the work related to the self-assembly (Wang et al. 2004, Gu et al. 2008, Qu et al. 2008, Thompson et al. 2008) and drug delivery properties of amphiphilic graft copolymers (van Krevelen 1997, Francis et al. 2003, Cheng et al. 2006). Unlike those block copolymer micelles (Mahmuda et al. 2008, 2009), it is noteworthy that the pendant group of graft copolymers could be the only hydrophobic moiety that will form the hydrophobic microdomains and contribute to the major interaction with the hydrophobic drug molecules. Therefore the investigation on the impact of hydrophobic pendant groups of amphiphilic graft copolymers on the solubility enhancement of hydrophobic drugs will be helpful to explore the rationale of the structural compatibility on drug solubilisation. In this work, Poly-l-lysine (PLL) is used as the hydrophilic backbone to

graft alkyl (C_{16}) chains and cholate pendant groups respectively (Scheme 1). Two sterol drugs with different water solubility (prednisolone and estradiol) (Scheme 2) were used as "cholate like" model drugs for the cholate grafted PLL to compare the solubilisation with the alkyl grafted PLL. Alkyl chains are common hydrophobic groups for fabricating amphiphilic graft copolymers and low molecular amphiphiles. Meanwhile, a non sterol-like drug, griseofulvin, were also selected for a comparison from the solubilisate side.

Experimental

Materials

Poly-L-lysine (PLL) (MW = 15k - 30k Da), cholic acid (CA), palmitoyl chloride, dicyclohexyl carbodiimide (DCC), N-hydroxysuccinimide (NHS), deuterated dimethyl sulfoxide (DMSO-d_6), prednisolone, estradiol, and griseofulvin were purchased from Sigma-Aldrich, USA. High performance liquid chromatography (HPLC) grade solvents were obtained from Sigma-Aldrich, UK. Other solvents and compounds were all obtained form Beijing Chemical Reagents Company, China. All reagents were used as received.

Synthesis of cholate grafted PLL (PLL-CA)

The PLL-CA was synthesized as previously described (Gu et al. 2008). Typically, cholic acid (10 g, 24 mmol, 1 equiv) and DCC (5 g, 24 mmol) were dissolved in 50 mL of dimethyl sulfoxide (DMSO). To this was added 3.4 g (30 mmol, 1.25 equiv) of NHS with stirring. The mixture was stirred for 15 h at room temperature then filtered. The filtrate was precipitated by n-hexane (200 mL), washed and vacuum dried to yield 11.2 g (90%) of cholic acid succinimide ester. MALDI-TOF MS: $[M^+] = 505.2$ (Calculated: $[M^+] = 505.68$).

To 15 mL of DMSO solution containing PLL (0.2 g, 0.96 mmol of lysine segment) and triethyl amine (TEA, 0.35 mL) was added dropwise the desired amount of the cholic acid

succinimide ester (0.1-0.4 mmol) in 10 mL of DMSO with stirring. The reaction mixture was stirred for 24 h at room temperature, precipitated and washed with diethyl ether, then dispersed in water for dialysis (Molecular weight cut off 12k Da) against distilled water (5 L with 6 changes in 24 h) and freeze dried to obtain fibre-like PLL-CA with 62-65% yield (calculated as % of the starting polymer weight). The cholate grafting level was determined by elemental analysis (Thompson et al. 2008).

Synthesis of palmitate grafted PLL (PLL-PAL)

PLL (0.2 g, 0.96 mmol of lysine segment) was dissolved in 20 mL of DMSO. To this was added 0.35 mL of TEA and desired amount of palmitoyl chloride (30-160 μL) with violent stirring. The mixture was stirred for 24 h at room temperature, protected from light, and dialysed (Molecular weight cut-off 12k Da) against ethanol/water (4:1, 2 L, six changes within 24 h) and then water (5L, six change within 24 h), and freeze-dried to gain yellow colour product with the yield in a range of 61-71%.

Preparation of polymer dispersion

Polymers (PLL-CA or PLL-PAL) were dispersed in water at desired concentrations by probe sonication using a JY96-II probe sonicator (Zhejiang Xin-Zhi, China) with the output set at 150 W.

Drug solubilisation

Drug loading was achieved by probe sonicating desired amount of drug (prednisolone, estradiol or griseofulvin) in the polymer dispersions (1 mg/mL or 3 mg/mL) as prepared above for 5 min with the maximum output (Cheng et al. 2006, Qu et al. 2006). For all drugs,

the drug to polymer initial weight ratios of 2:1 and 5:1 were used. Drug levels contained in the polymeric micelles were measured using HPLC.

For polymer/prednisolone and polymer/estradiol formulations, after sonication the dispersions were cooled down to room temperature and equilibrated for 4 h, then were filtered through syringe filters (pore size: 450 nm with prefilters). The filtrates were then diluted with the mobile phase (water/acetonitrile 64:36), and was injected (20 μL) into a reverse phase 3.5 μm C18 symmetry column (4.6 × 75 mm, Waters Instruments, U.K.) at a mobile phase flow rate of 1 mL/min. The HPLC consists of a Waters 515 isocratic pump and a Waters 717 autosampler, and sample detection was achieved using a Waters 486 variable wavelength ultraviolet wavelength detector (λ = 243 nm for prednisolone and 205nm for estradiol). The retention peak was 3 min and 10 min respectively for prednisolone and estradiol. The drug contents in the samples were quantified by comparing to a standard calibration containing the drugs dissolved in the mobile phase (6 μg/mL - 25 μg/mL), R^2 = 0.999.

The above procedure was repeated for polymer/griseofulvin formulations but using a different mobile phase (45:55 v/v of acetonitrile: 45mM potassium dihydrogen phosphate in water and adjusted to pH=3 with orthophosphoric acid). The UV detection wavelength was 293 nm and the column used was a RP Phenomenex C18 (250mm × 46mm, 5μm). Calibration graphs were constructed to determine the drug content in the samples where griseofulvin was dissolved in the mobile phase (0.6 μg/mL - 10 μg/mL), R^2 = 0.999. The retention peak was detected at 9.5 min.

The amount of drugs in the polymeric micelle system in relation to the drug solubility in water, also referred to as solubility enhancement of the drugs in polymer solution, was calculated as following:

$$\text{Solubility enhancement} = 100\% \times [\text{Drug}]_{\text{dispersion}}/[\text{Drug}]_{\text{water}} \quad (1)$$

where the $[Drug]_{dispersion}$ and $[Drug]_{water}$ are the concentrations of the drug in polymer solution and the aqueous solubility of the drug detected by HPLC.

Elemental Analysis

The contents of C, H and N of the polymers were detected using a Perkin Elmer 2400 analyser. The hydrophobic pendant grafting level of PLL-CA and PLL-PAL was evaluated based on the method reported in the previous works (Gu et al. 2008, Qu et al. 2008, Thompson et al. 2008), which was calculated by the comparison of the C/N ratio of the graft polymer to the parent PLL.

^{1}H NMR analysis

^{1}H NMR analysis of synthesized polymers was performed on polymer solutions in DMSO-d_6 using a Bruker AMX 600 MHz spectrometer.

Size measurements

Particle size measurement was carried out for all polymer/drug formulations and polymer alone in water with a NanoPlus Zetasizer (Malvern Instruments, UK). All samples were passed through membrane filters (pore size: 450 nm, Millipore) before measurement. The measurement was conducted in triplicates.

Transmission electron microscopy

Carbon-coated 200 mesh copper grids were discharged and sample dispersions applied, followed by the application of phosphate-tungstic acid (1%) for negative stain. The grids were dried and imaged using a LEO 902 transmission electron microscope (TEM) at 80 kV.

X-ray powder diffraction

Wide-angle X-ray diffraction (WAXD) of powder specimen (5 mg) was obtained using a wide-angle Rigaku D/max-2500 diffractometer (Rigaku Corporation, Japan) with Cu-Kα radiation (50 kV × 250 mA). The samples were prepared using following methods: (1) Selected formulations (polymer concentration: 1 mg/mL, drug to polymer initial ratio: 2:1 w/w) as-prepared in 2.4.3 were freeze-dried; (2) Formulations were prepared via probe sonicating the drug/polymer solution with a drug to polymer initial ratio of 0.4:1 w/w, and freeze-dried without filtration; (3) Drug and polymer were blended as control samples.

Infrared spectroscopy (FTIR)

Infrared spectroscopy of freeze-dried polymers or polymer/drug formulations prepared as the same procedures described in the XRD measurements was recorded using a Brukerequinox 55 FTIR spectrometer. The samples were pressed with KBr under vacuum and scanned from 4000 to 400 cm^{-1} with a resolution of 2 cm^{-1}.

Drug-polymer compatibility calculations

The compatibility between drug and hydrophobic pendant group of the amphiphilic graft polymers was calculated by the Flory-Huggins interaction parameter (χ_{sc}) using the Hildebrand-Scatchard equation (eq.2).

$$\chi_{sc} = (\delta_s - \delta_c)^2 \frac{V_s}{RT} \quad (2)$$

where δ_s and δ_c are the solubility parameters of drug (s) and hydrophobic pendant group respectively . V_s is the molar volume of the drug, R is the gas constant, and T is the Kelvin temperature. The solubility parameter (δ) of the drug (δ_s) and the hydrophobic pendant group of the polymers (δ_c) was obtained separately by group contribution method (GCM) as

described by van Krevelen (van Krevelen 1997), which uses partial solubility parameters to calculate the total solubility parameter as outlined in eq. 3.

$$\delta = \left(\delta_d^2 + \delta_p^2 + \delta_h^2\right)^{1/2} \quad (3)$$

where δ_d, δ_p, and δ_h are the partial solubility parameters indicating contributions from Van der Waals, polar interactions, and hydrogen bonding between molecules respectively. Each individual component can be calculated according to the following equations (eq. 4-6).

$$\delta_d = \sum \frac{F_{di}}{V} \quad (4)$$

$$\delta_p = \frac{\left(\sum F_{pi}^2\right)^{1/2}}{V} \quad (5)$$

$$\delta_h = \left(\sum \frac{E_{hi}}{V}\right)^{1/2} \quad (6)$$

where F_{di} and F_{pi} are the molar dispersion and polar attraction constants, respectively, and E_{hi} is the hydrogen bonding energy.

Statistical analysis

Data represent at least three independent experiments and are expressed as mean ± SD. Statistical significance was assessed using Student's t-test using SPSS software. The difference was considered to be statistically significant if the probability value was less than 0.05 ($p<0.05$).

Results and Discussion

Polymer synthesis and self-assembly

The synthesis and characterization of PLL-CA has been described previously (Gu et al. 2008). PLL-PAL was synthesized by reacting PLL with palmitoyl chloride in the presence of

trimethyl amine. The structure of the products was examined using ^{1}H NMR. The proton assignments for the PLL-PALs dissolved in DMSO-d_6 were as follows: δ4.3 ppm = CH (PLL), δ3.2-3.4 ppm = CH_2-N^+ (PLL) and CH_2-N-CO (palmitoyl), δ2.2 ppm = CH_2-CO-N (palmitoyl), δ1.2-1.8 ppm = CH_2 (PLL and palmitoyl), δ0.9 ppm, = CH_3 (palmitoyl). The palmitoylation level of PLL-PALs was evaluated using elemental analysis, by comparing the carbon to nitrogen molar ratio of PLL and the PLL-PALs, which were also confirmed by comparing the ^{1}H NMR integrals of palmitoyl proton peak at 0.9 ppm and the PLL proton peak at 4.3 ppm. The details of chemical composition of the PLL-CAs and PLL-PALs synthesized for this study are summarized in Table 1. By changing the initial feed ratio of reagents, the CA and palmitoylation level could be adjusted in a wide range, i.e. 10-50 mol% to the lysine segment.

The PLL grafted with two different hydrophobic pendant groups, i.e. PLL-CA and PLL-PAL, self-assembled in aqueous solution after probe sonication due to the amphiphilicity nature of the polymers, irrespective of the structure of pendant groups. Previous studies have proven that the supramolecular structures formed by amphphilic graft copolymers transform from polymeric micelle to solid nanoparticle with the increase of hydrophobicity (Wang et al. 2004, Qu et al. 2008). Polymeric micelle will be formed by graft copolymers with relatively low level of hydrophobic grafting, e.g. less than 40 mol%. In this work, the dispersions formed by the polymers in water were stable for at least 24 h, except the PLL-PAL50, having the highest palmitoylation level, which precipitated within 12 h. TEM images showed spherical particles for PLL-CAs (Thompson et al. 2008) while similar spherical nano-sized aggregates were also found with PLL-PALs (Figure 1). It is revealed that PLL-PAL50 self-assembled into nanoparticle while the other PLL-PAL copolymers formed polymeric micelles (Qu et al. 2006, 2008, Gu et al. 2008). The hydrodynamic diameter of the PLL-CAs and PLL-PALs aggregates is listed in Table 1. A size over 200 nm possibly indicates a multi-core

structure of the aggregates rather than traditional micelles formed by small molecule surfactants, which have been observed in both block copolymer and graft copolymer systems (Borisov and Halperin 1996, Uchegbu et al. 2001, Hsu et al. 2005).

Drug solubilisation

It has been well documented that the grafting level influences the hydrophobicity of the copolymer and thus will have an impact on the drug incorporation (Cheng et al. 2006, Qu et al. 2006). Therefore, a series of PLL-CAs and PLL-PALs with different grafting levels were used for the drug solubilisation studies. The drug solubilisation studies were carried out by mixing the drugs in polymer solution followed by probe sonication (Cheng et al. 2006). The solvent evaporation method was not used in the preparation of drug formulations to eliminate the influence of residue solvent on the polymer-drug interactions (Yokoyama et al. 1998). Three hydrophobic model drugs were selected. As shown in Scheme 1 and 2, the chemical structure of the cholate pendant group of PLL-CA is similar to that of prednisolone and estradiol. Prednisolone and estradiol are sterol drugs but have different octanol-water partition coefficient (Log P) (Table 2). Griseofulvin does not exhibit similar chemical structure as prednisolone or estradiol, however the physicochemical properties such as the molar volume and Log P is similar to prednisolone, i.e. 353 g/mol and Log P of 2.2 for griseofulvin and 360 g/mol and Log P of 1.8 for prednisolone (Nielsen et al. 2001, Dahan et al. 2007).

Figure 2 summarizes the drug level incorporated by the amphiphilic graft copolymers at two different polymer concentrations with different drug to polymer initial weight ratios. In general for each polymer/drug formulation, the amount of drug detected by HPLC increases with the increase of polymer concentration, i.e. from 1 to 3 mg/mL, and the drug to polymer initial mass loading ratio, i.e. from 2:1 to 5:1 (w/w), indicating drug solubilisation by the

polymeric aggregates (Kwon et al. 1997, Shim et al. 2006). As shown in Figure 2, the solubilisation of prednisolone and estradiol is significantly dependent on the chemical structure of the amphiphilic copolymers. The PLL-CAs resulted in a much greater prednisolone solubilisation, with a maximum solubilisation of 0.95 mg/mL, ca. 4-fold greater than the aqueous solubility of the drug (250 μg/mL, HPLC) (Figure 2a). However, only a maximum 30% increase of prednisolone concentration (0.33mg/mL /mL) is found with PLL-PAL/prednisolone formulations. Similarly, although the detected estradiol concentration in all formulations was relatively low, which was probably due to the very low water solubility, i.e. 3.5 μg/mL, a maximum 48-fold increase of estradiol solubility was obtained with PLL-CA32 (170 μg/mL) (Figure 2b), 6 times higher than that in the PLL-PAL formulations (with a maximum drug concentration of 30 μg/mL).

Unlike prednisolone, the solubilisation of griseofulvin in PLL-CAs and PLL-PALs polymeric micelles is independent of the chemical structure of the polymers. Poor griseofulvin solubilisation was observed irrespective of the type of hydrophobic pendant groups and the grafting level (Figure 2c). Maximum solubilisation (219 and 174 μg/mL), only 2-fold of the drug's water solubility (91 μg/mL, HPLC), was achieved respectively with PLL-CA and PLL-PAL when the highest drug loading ratio (5:1) and polymer concentration (3 mg/mL) were used. This is in good agreement with other published work using amphiphilic block copolymers containing linear poly(lactic acid) and poly(butylene oxide) hydrophobic blocks (Pierri et al. 2005, Ribeiro et al. 2009), although we used lower polymer concentration with a higher drug to polymer mass ratios.

Physicochemical characterization to evaluate the interactions between drug and polymer

Prednisolone and griseofulvin formulations were selected for comparison since the two drugs have similar Log P, molecular weight and molar volume. It seems that the incorporation efficiency of drugs influences the size and morphology of the micelles. As seen in Table 1, the PLL-PAL/prednisolone formulations have smaller hydrodynamic size when compared to unloaded PLL-PAL self-assemblies, especially the formulations with higher hydrophobic grafting levels, i.e. PLL-PAL28 and PLL-PAL50. For PLL-CA/prednisolone formulations, the level of hydrophobic grafting also has an impact on the hydrodynamic size of drug loaded micelles. While the drug loaded PLL-CA10 displays a much larger hydrodynamic size, increasing the level of grafting has resulted in a sequential reduction of particle size for PLL-CA15 and PLL-CA32 formulations. The results suggest that the interaction between the drug and the pendant groups is the predominant driving force for prednisolone solubilisation, which resulted in the formation of a more compact core structure upon the increase of hydrophobic grafting (Huang et al. 1998, Jiang et al. 2006). However for both PLL-CA and PLL-PAL/griseofulvin formulations, the type of pendant groups of the polymers has no impact on the hydrodynamic size where increasing the grafting level did not change the size significantly compared to unloaded polymeric self-assemblies (Table 1). This also corresponds to a low level of solubilisation across all levels of grafting (Figure 2c). The morphology of PLL-CA and PLL-PAL formulations are shown in Figure 3. Similar spherical particles are seen for the PLL-PAL28/prednisolone formulation and the griseofulvin formulations of PLL-PAL28 and PLL-CA32 (Figure 3a-c), whereas irregular shaped particles are observed in the PLL-CA32/prednisolone formulation (Figure 3d).

Powder X-ray diffraction was used to further evaluate the polymer-drug interaction, as well as the state of the drug in the polymeric micelles. The XRD patterns of the drugs and their formulations are plotted in Figure 4. It is revealed that the two drugs are highly crystallized (Figure 4) and the freeze-dried polymers were in amorphous state (data not

shown). As shown in Figure 4a, the crystal peaks are visible in all freeze-dried griseofulvin/polymer formulations, however compared to the drug-polymer blend (curve 2), the unfiltered samples (curve 3 and 5) with a fixed drug content (40 wt%) have weaker intensities despite free drugs might be present in the aqueous phase. This suggests that the presence of polymer decreased the crystallinity of griseofulvin in the polymer dispersions. For filtered samples (Figure 4a, curve 4 and 6), it is expected that most of the drug molecules would be solubilised in the micelle cores. However the appearance of crystal peaks in the filtered samples could indicate that the interaction between the pendant groups, i.e. cholate or palmilate, and griseofulvin is not adequate to physically stabilize the drug molecules at molecular level, although it might have reduced the rate of drug crystallization. Stronger intermolecular interaction between drug molecules rather than that between the drug and polymer might cause drug crystallization and hence inefficient drug incorporation in the polymeric micelles (Liu et al. 2004). This is evident from the griseofulvin solubilisation data (Figure 2c).

On the other hand, Figure 4b (curve 4) reveals very weak diffraction patterns corresponding to prednisolone crystal in the filtered prednisolone/PLL-CA32 sample (drug/polymer feed ratio = 2:1 w/w) which contains ca. 45 % (w/w) of the drug (HPLC). Comparably the freeze-dried sample of the unfiltered formulation with an apparent 40 % (w/w) drug content (drug/polymer = 0.4:1 w/w), thus with less drug in the micelle core comparing to the filtered one, has much stronger crystal peaks (Figure 4b, curve 3), which is comparable to that of the drug-polymer blend (0.4:1 w/w) (Figure 4b, curve 2). The results indicate that the cholate pendant group and prednisolone have formed stronger interaction to stabilize the incorporated drug molecules, because the amorphous drugs were more likely incorporated inside the PLL-CA32 micelles. Weak crystal diffraction peaks are also observed in the filtered prednisolone/PLL-PAL28 sample (Figure 4b, curve 6), however the low drug

content in this formulation, i.e. 74 μg of drug with ca. 1 mg of polymer (HPLC) must be taken into consideration compared to prednisolone/PLL-CA32.

The FTIR spectra in Figure 5a demonstrate a new absorbance at 1100 cm^{-1} in the prednisolone/PLL-CA32 formulations and the intensity increases with the drug loading level (curve 3 and 4). Such absorbance is not found in prednisolone/PLL-CA32 blend (Figure 5a, curve 5) and similarly no extra band was observed in the prednislone/PLL-PAL28 (Figure 5b) and all griseofulvin formulations (Figure 5c). The results further confirm that the PLL-CA could strongly interact with prednisolone possibly due to structural similarity between the cholate group and prednisolone molecules.

Calculation of the compatibility between polymer and drug

With the assumption that that the drugs majorly interact with the hydrophobic pendants (Liu et al. 2004), Flory-Huggins interaction parameters (χ_{sc}) between the drugs and the pendant groups of the polymers are calculated and the results are listed in Table 2. Comparing the compatibility of the two polymers with the drugs, the calculated χ_{sc} for PLL-CA between the cholate pendant group and griseofulvin, prednisolone and estradiol is all smaller than that for the PLL-PAL counterparts. In comparison with the experimental results as shown in Figure 2, the solubilisation of prednisolone and estradiol is indeed found greater with the PLL-CA micelles, in good agreements with the χ_{sc} prediction (Figure 2a, b), whereas the difference in griseofulvin solubilisation capacity of the two different types of polymers, i.e. PLL-CA and PLL-PAL, is not obvious (Figure 2c). However, it is also noted in Table 2 that while HPLC results show that PLL-CA copolymers have better solubilising capacity for prednisolone than griseofulvin (Figure 2a and c), the χ_{sc} of griseofulvin with the pendant group of PLL-CA is even smaller than that of prednisolone. This demonstrates the limit of

using χ_{sc} to predict the drug solubilisation in particular, when the drug and the hydrophobic pendant group of amphiphilic copolymer both have similar sterol-like structures.

As mentioned above, the XRD and FTIR data imply stronger interaction between prednisolone and the PLL-CAs, which is presumed to be stronger hydrogen bondings formed. The contribution of H-bonding on the drug solubilisation can be evaluated by comparing the partial solubility parameters between the drugs and the pendant groups of the polymers, i.e. $\Delta\delta_d$, $\Delta\delta_p$ and $\Delta\delta_h$, because the enthalpy of mixing (ΔH_M) can be calculated using the following equation (Liu et al. 2004):

$$\Delta H_M = \phi_s \phi_c (\Delta\delta_d^2 + \Delta\delta_p^2 + \Delta\delta_h^2) \quad (7)$$

where ϕ_s, ϕ_c are volume fractions of the drug and polymer. From Table 2 the δ_d and δ_p values are very similar; meanwhile the δ_h of predinsolone and griseofulvin are different. Therefore it can be calculated that the $\Delta\delta_h$ between prednisolone and the cholate is much lower, which leads to the decrease of mixing enthalpy between prednisolone and PLL-CA micelles.

Conclusions

PLL amphiphilic graft copolymers with hydrophobic palmitate and cholate pendant groups were synthesized. Their ability to solubilise sterol drugs was tested. It is shown that the chemical structure of the hydrophobic pendant group of the amphiphilic graft copolymers has significantly influenced the solubilisation of poorly water-soluble drugs. With “drug-like” pendant groups, PLL-CA achieved higher prednisolone and estradiol encapsulation than the palmitoylated PLL (PLL-PAL) despite the water solubility of these two drugs is very different. Besides, the PLL-CA also has higher solubilisation capacity for prednisolone when comparing with griseofulvin, a non-steroidal drug with similar molecular weight and Log P, although the calculated χ_{sc} between griseofulvin and the cholate is lower. This work provides valuable information not only to understand the contribution of structural compatibility on the

drug-polymer interactions but also benefit the future design of amphiphilic graft copolymers as hydrophobic drug solubilisers.

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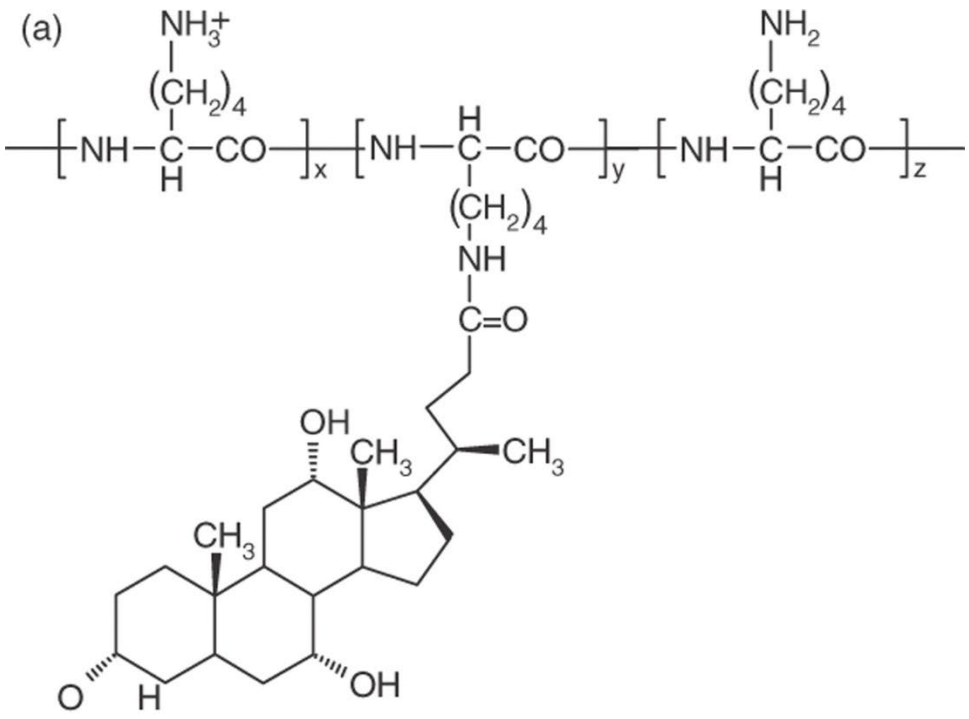
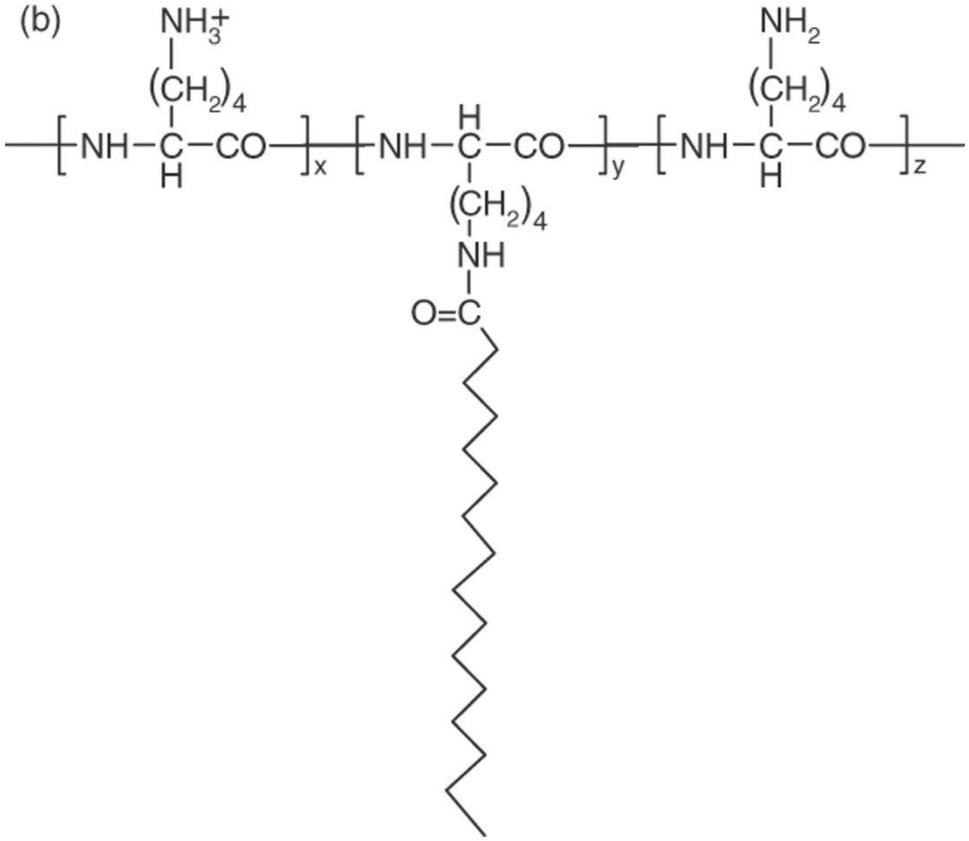
Scheme 1. Chemical structure of PLL-CA (a) and PLL-PAL (b).





Scheme 2. Chemical structure of prednisolone (a), estradiol (b), griseofulvin (c).

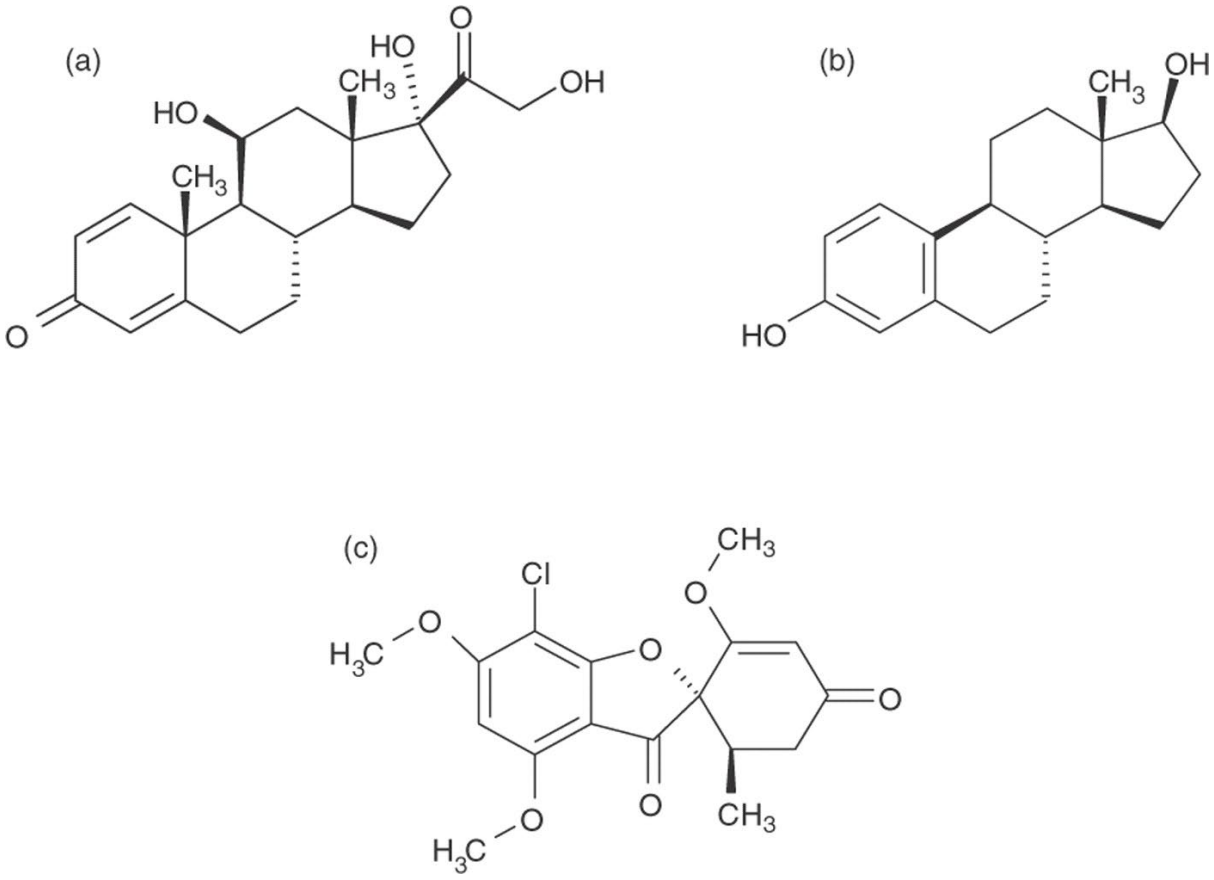


Table 1. Synthesis of PLL-CA and PLL-PAL and the hydrodynamic diameter of drug loaded polymeric micelles in aqueous solution.

	Initial feed ration (mol%)[a]		**Grafting level (mol%)[b]**		**Yield (%)**			
	CA	**PAL**	**CA**	**PAL**		**Z-ave. size of unloaded polymeric self-assemblies (nm)**	**Z-ave. size of griseofulvin loaded micelle[c] (nm)**	**Z-ave. size of prednisolone loaded micelle[c] (nm)**
PLL-CA10	13	-	10	-	62	238 ± 5.6	258 ± 18	1270 ± 93
PLL-CA15	18	-	15	-	65	188 ± 15	253 ± 16	242 ± 10
PLL-CA32	35	-	32	-	64	259 ± 11	235 ± 14	176 ± 23
PLL-PAL10	-	13	-	10	61	266 ± 47	205 ± 24	244 ± 44
PLL-PAL28	-	35	-	28	65	539 ± 54	483 ± 80	225 ± 44
PLL-PAL50	-	55	-	50	71	442 ± 29	499 ± 38	222 ± 45

Notes: [a]Mole of CA or palmitoyl chloride fed to 100 mole of lysine unit. [b] Number of CA or palmitoyl chloride group per 100 lysine units. [c] Z-average mean hydrodynamic diameter measured using DLS at a fixed polymer concentration of 3 mg/mL in water with the drug/polymer loading ratio = 2:1 (n = 3, mean ± s.d.).

Table 2. Solubility parameter of drugs and the hydrophobic moieties of the copolymers.

	MW	logP	V (cm^3/mol)	δ_d	δ_p	δ_h	δ	$X^b_{sc/CA}$	$X^b_{sc/PAL}$
Griseofulvin	352.5	2.2	230.1	20.38	8.84	8.44	24.20	0.051	4.59
Prednisolone	360	1.8	242.1	21.52	8.88	16.26	28.40	1.16	12.28
Estradiol	272	3.57[a]	208.3	20.55	4.80	13.86	25.24	0.0075	5.47
Cholate	392	–	302.8	19.65	5.57	14.30	24.94	–	–
Palmitate	239	–	253.6	16.64	3.04	2.81	17.15	–	–

Notes: [a]Data cited from http://www.drugbank.ca. [b] Flory-Huggins interaction parameter (T = 300 K) between drug and hydrophobic pendant group of PLL-CA (χsc/CA) and PLL-PAL (χsc/PAL).

Figure 1. TEM images of aggregates formed by PLL-PAL28 (a) and PLL-PAL50 (b), prepared from aqueous dispersion at a polymer concentration of 3 mg/mL.

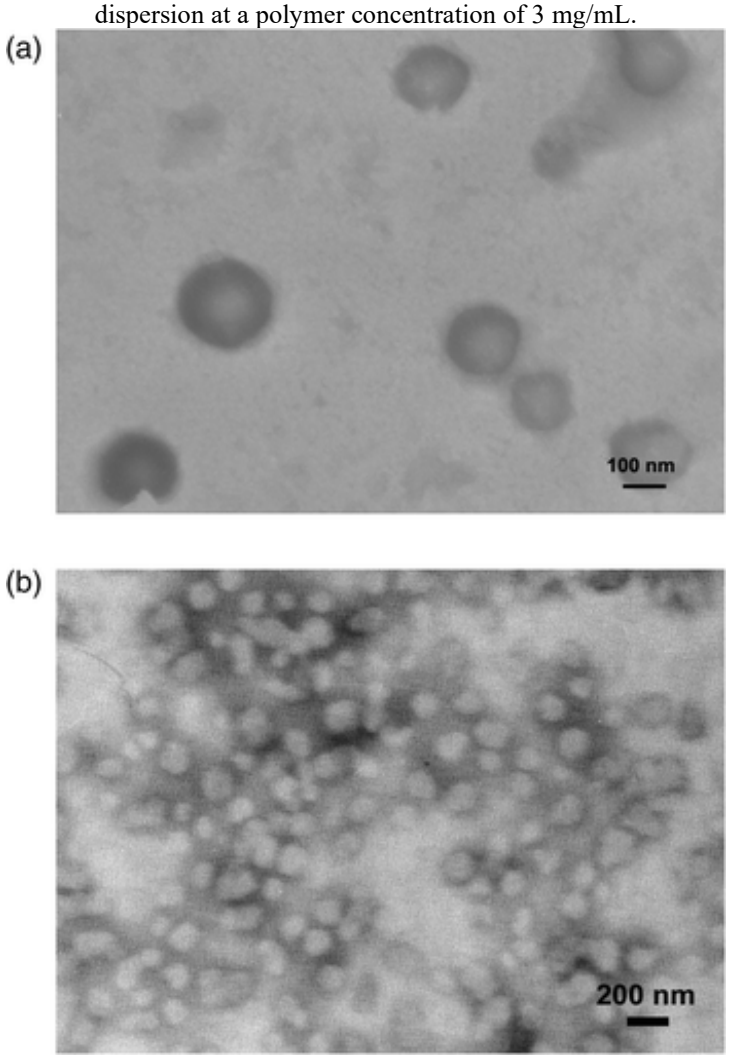


Figure 2. Solubilisation and solubility enhancement of prednisolone (a), estradiol (b) and griseofulvin (c) in PLL-CAs and PLL-PALs with different polymer concentration and drug/polymer loading ratio. Filled bars: Solubility; Open bars: Solubility enhancement against aqueous drug solubility in water. *p<0.05, **p<0.01 indicate that the drug solubility and solubility enhancement show significant difference between PLL-CA and PLL-PAL at the addressed polymer concentration and drug-loading level.

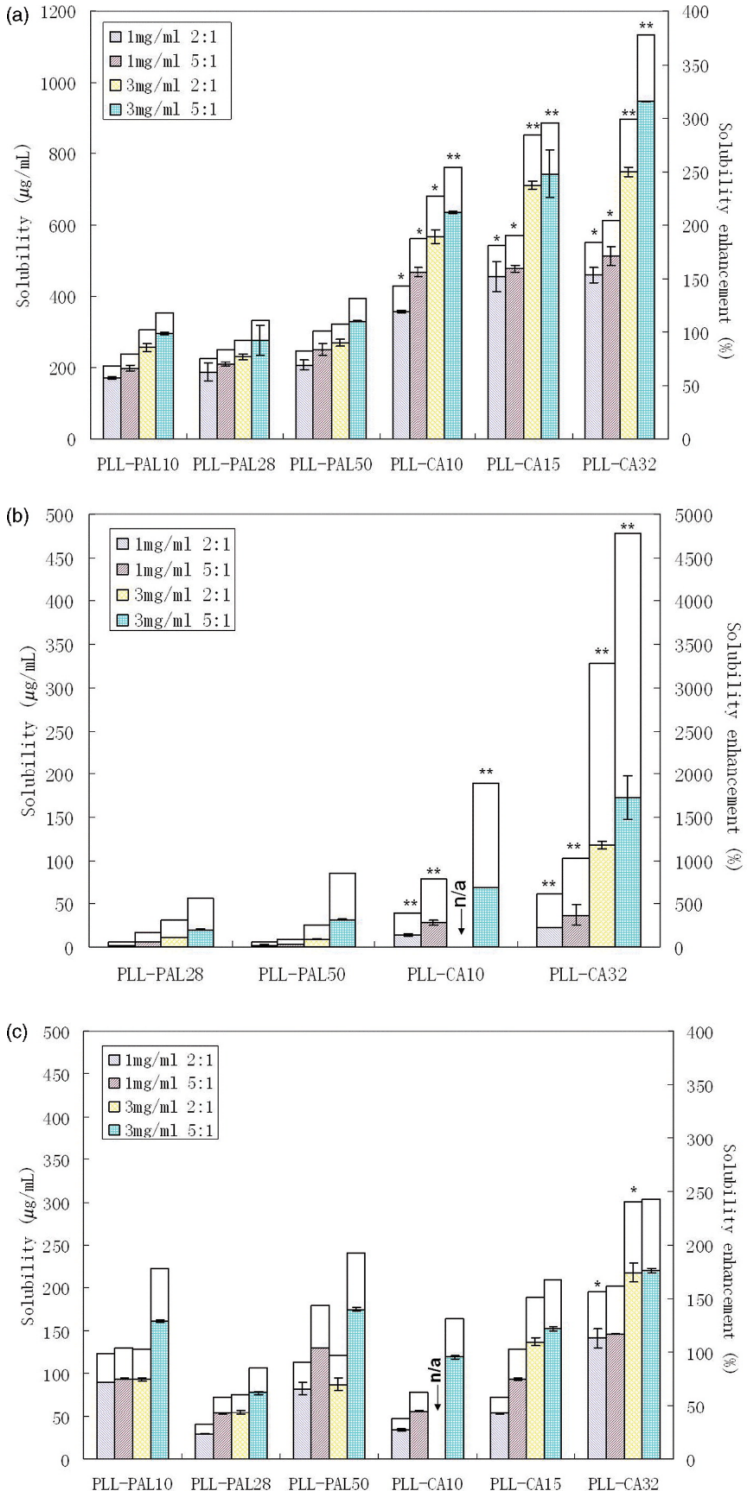


Figure 3. TEM images of griseofulvin loaded (a) and prednisolone loaded (b) PLL-PAL28, and griseofulvin loaded (c) and prednisolone loaded PLL-CA32 formulations (d) prepared from filtration of probe-sonicated polymer/drug dispersions at a polymer concentration of 3 mg/mL with a drug/polymer feed ratio of 2:1.

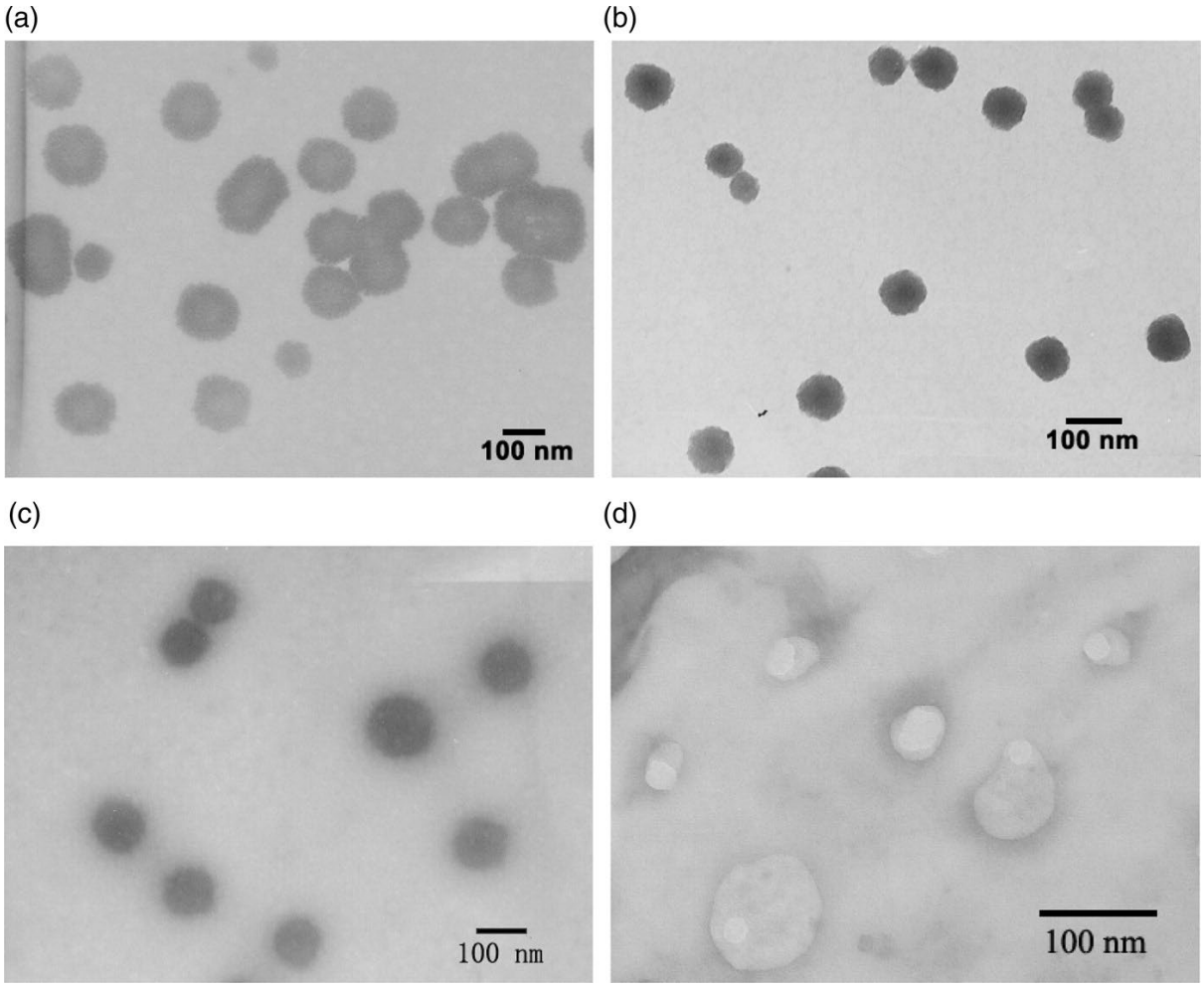


Figure 4. XRD patterns of griseofulvin loaded (a) and prednisolone loaded PLL-CA32 and PLL-PAL28 (b).

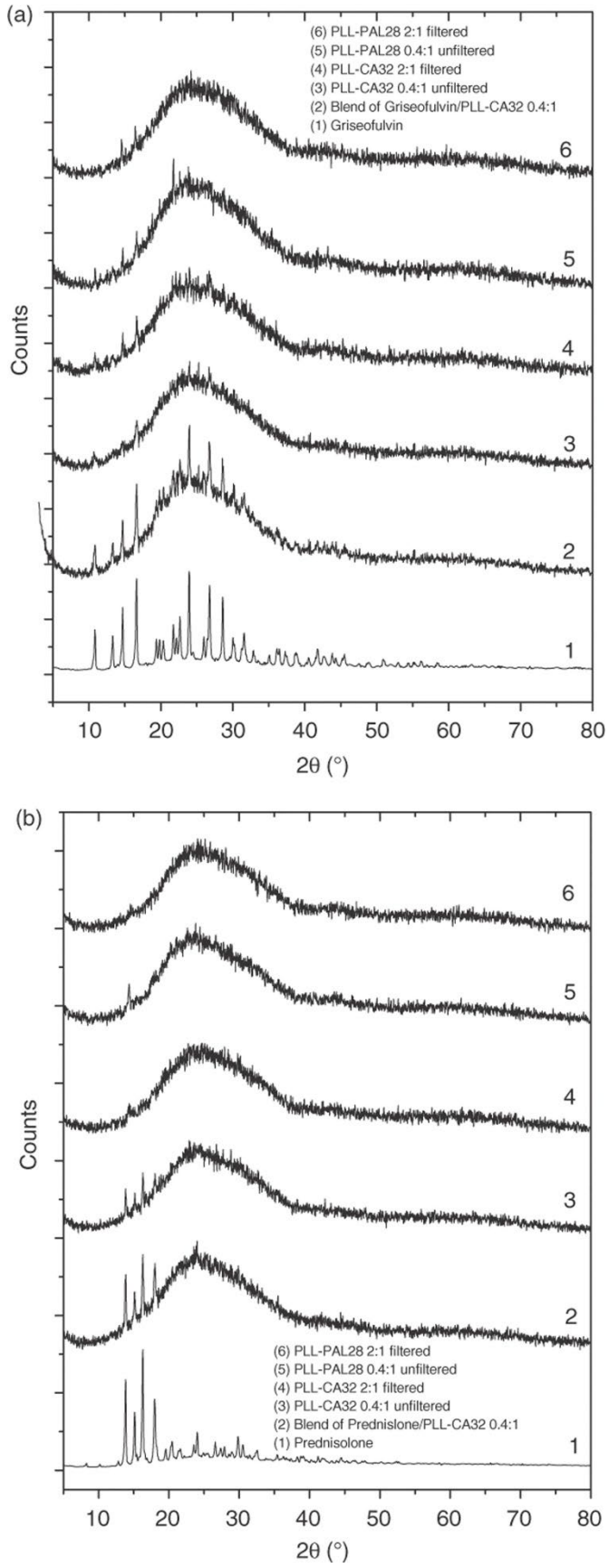


Figure 5. FTIR spectra of prednisolone loaded PLL-CA32 (a), prednisolone loaded PLL-PAL28 (b) and griseofulvin loaded PLL-CA32 and PLL-PAL28 (c).