Colloids and Surfaces A: Physicochem. Eng. Aspects 429 (2013) 24–30

Contents lists available at SciVerse ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

journal homepage: www.elsevier.com/locate/colsurfa

Preparation and *in vitro* evaluation of apigenin-loaded polymeric micelles

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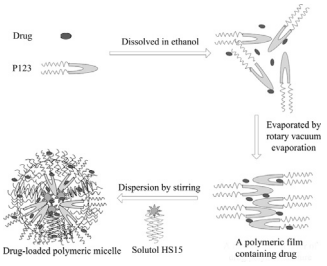
HIGHLIGHTS

- A novel apigenin-loaded mixed micelle was prepared and optimized.
- The solubility of apigenin in the micelles was 148 times that of crude apigenin.
- CMC of the mixed micelles was as low as 4.23×10^{-5} mol/L.
- The cytotoxicity against HepG2 and MCF-7 cells shows enhanced inhibition rate.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 22 December 2012
Received in revised form 2 March 2013
Accepted 22 March 2013
Available online 1 April 2013

Keywords:
Micelle
Apigenin
Pluronic P123
Central composite design

ABSTRACT

The objective of this study was to prepare apigenin-loaded polymeric micelles to improve the solubility of apigenin in water. The polymeric micelles composed of Pluronic P123 and Solutol HS 15 were prepared by a thin-film dispersion method and the formulation was optimized with a central composite design (CCD). The obtained micelles were spherical under transmission electron microscope (TEM) with an average diameter of 16.9 nm, the entrapment efficiency and drug loading was 96.36% and 1.32%, respectively. The *in vitro* drug release study showed nearly 84% of apigenin was released from micelles within 36 h, showing the sustained release property. Cell toxicity assay indicated that the cytotoxicity of apigenin-loaded polymeric micelles against HepG2 and MCF-7 cancer cell *in vitro* was remarkably higher than that of the free drug. All the results provided the evidence for the great potential of polymeric micelles as nanocarrier for apigenin as a poorly water soluble drug.

1. Introduction

Flavonoids are a group of phenolic plant pigments and common constituents of human diet as well, presenting in most fruit and vegetables [1]. They are considered as dietary for a function of anti-cancer and anti-oxidant [2]. In several cases, flavonoids have been reported to possess anti-cancer potential [3,4]. Apigenin (4′,5,7-trihydroxy-flavone) as a common flavonoid is believed to possess the preventive and therapeutic potential against cancers. It has been shown to possess anti-inflammatory, free radical scavenging properties with superiority of non-mutagenic and low toxicity [5]. More importantly, according to previous studies, apigenin could induce apoptosis of cancer cells and exert effects on inhibiting the invasive process [6]. However, the solubility of apigenin as a poorly water soluble drug is only 2.16 μg/mL in water [7] and 0.001–1.63 mg/mL in high hydrophilic or nonpolar solvents [8], leading to a poor absorption in gastrointestinal tract. Therefore, the improvement in solubility and bioavailability is urgently needed for development and application of apigenin.

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http://dx.doi.org/10.1016/j.colsurfa.2013.03.051

Over the past two decades, nanoscale micelles have demonstrated great potential in delivering anticancer drugs [9,10]. Polymeric micelle has a particle size ranging from 10 to 100 nm, preventing from being recognized by reticuloendothelial system (RES), increasing the systemic circulation time. On the other hand, the small size (<100 nm) of micelles allows for their efficient accumulation in tumor tissues *via* the enhanced permeability and retention (EPR) effect. Moreover, polymeric micelle has many other special properties such as biocompatibility and increased stability. Conventionally, this kind of micelle was composed of amphiphilic block copolymers. The hydrophobic part may face to face to form a hydrophobic core while the hydrophilic chains crosslinking outside forms a shell when the block copolymer self-assembles in water [11]. Consequently, the hydrophobic drugs can easily be encapsulated into the hydrophobic cores of the polymeric micelles.

P123 is one of the most common representatives of amphiphilic block copolymers, whose structure is PEO–PPO–PEO [12]. The PPO part is hydrophobic, leading to an aggregation against water and providing a local hydrophobic microenvironment where the hydrophobic drug can be dissolved, while the hydrophilic PEO part maintains the dispersion stability of the formed micelles. In P123, the proportion of PPO part is as high as 70%, which means micelles formed by P123 should have sufficient capability for solubilizing the poorly water soluble drugs because of the large bulk volume of inner core. However, the proportion of PEO blocks in P123 is only 30%, forming a relative thin shell of the micelle with a poor dispersibility. In addition, almost 5.4% PEO blocks exist within the core of the micelles at a low temperature under 30 °C [13], which decreases the hydrophobicity of core as well as the hydrophility of shell of simple P123 micelles. The unbalanced ratio of the PEO and PPO chains in P123 results in the destabilization or unstability of the micelle made from this block copolymer [14]. Therefore, it is obliged to modify the deficiency of simple P123 micelles aiming for preferred micelle stability.

According to previous study, mixed micelles composed of two or more kinds of polymer manifest synergistic properties, such as increased micelle stability and more effective solubilization capacity, superior to those composed of the individual component [15]. Solutol HS15 (polyethylene glycol-660 hydroxystearate), consisting of polyglycol mono- and diesters of 12-hydroxystearic acid, which has been recorded in the European Pharmacopoeia, is recommended as non-ionic solubilizing agent to be added to injection solutions [16]. Particularly, nanoformulations made from Solutol HS15 may show a long circulating action for the presence of about 30% of free polyethylene glycol (PEG) in Solutol HS15 [17], PEG can increase stability of colloidal dispersion system and enhance blood circulation time by decreasing macrophage up-take and complement activation [18,19]. The composition of Solutol HS15 can make it form mixed micelle combined with other copolymer, and improve the related properties of the micelle made from this copolymer. For example, Li et al. fabricated a propofol-loaded mixed micelles formed from mPEG-PLA and Solutol HS15, and found the solubilization of propofol by the mixed micelles was more efficient than those made of mPEG-PLA alone [16]. Ma et al. developed docetaxel-loaded mixed micelles whose main constituents were Solutol HS 15 and lipid S100. A satisfactory encapsulation efficiency (87.6% ± 3.0%) was achieved and the areas under the curve ((0–6 h)) levels of docetaxel in blood and tumors were significantly higher in the mixed micelle group (15.9 ± 3.2 μg/mL, 601.1 ± 194.5 μg/g) than that in the docetaxel injection group (7.2 ± 1.7 μg/mL, 357.8 ± 86.2 μg/g), which showed that the mixed micelles significantly improved the bioavailability [20]. Based on the reports, we hypothesize that mixed micelles made from P123 and Solutol HS15 may allow for the higher drug encapsulation, the better stability and higher anticancer efficiency.

In the study, an apigenin-loaded polymeric micelle system with P123 and Solutol HS15 as mixed polymer carrier was prepared by a thin-film dispersion method, and the preparation process was optimized with a central composite design (CCD). In addition, the physicochemical properties and *in vitro* cytotoxicity of the optimized drug-loaded micelles were investigated.

2. Materials and methods

2.1. Materials

Apigenin was obtained from TianCao chemicals Co. (Hangzhou, Zhejiang), Pluronic F127, Pluronic F68, Pluronic P123 were purchased from Sigma-Aldrich Chemical Co. (St. Louis, Missouri, USA). Solutol HS 15 was provided by BASF Co. (Germany), PEG (2000)-PLA (2000) was brought from DaiGang Biological Engineering Co.Ltd (Jinan, Shandong). All other chemicals were of analytical purity and commercially available.

2.2. Preparation of apigenin-loaded polymeric micelles

The apigenin-loaded micelles were prepared with a thin-film dispersion method [21]. Briefly, copolymers at certain amount and 1 mg of apigenin were dissolved in 5 mL of ethanol to form a mixture in a 50 mL round-bottomed flask. The organic phase was then removed by rotary evaporation under reduced pressure at 40 °C for 20 min to yield a polymeric film containing drug on the wall inside the flask. The flask was placed in a vacuum dryer overnight to ensure that the residual ethanol was dried thoroughly. The resulting polymeric film containing drug was dispersed by adding 5 mL of 0.58% (w/v) Solutol HS15 (29 mg) aqueous solution with stirring at 800 rpm for 40 min. Eventually, a clear and homogeneous micelle solution was obtained from the supernatant after centrifugation at 4000 rpm for 10 min.

2.3. Encapsulation efficiency and drug loading

In consideration of the poor water solubility of apigenin, the amount of free apigenin in the formulation was negligible. For this reason, the drug in the preparations was nearly equal to that entrapped into the micelles. Briefly, 0.1 mL of the original micelle suspensions was disrupted with 10 mL of ethanol, and the amount of entrapped apigenin was determined at 336 nm on an ultraviolet spectrophotometer. EE% and DL% were calculated as following:

$$EE\% = \frac{W_{\text{loaded}}}{W_{\text{added}}} \times 100\%$$

$$\text{DL}\% = \frac{W_{\text{loaded}}}{W_{\text{total}}} \times 100\%$$

where W_{loaded} is the apigenin amount entrapped into micelles, W_{added} is the initially added amount of apigenin in the formulation preparation, and W_{total} represents the amount of both drug and excipients in the whole formulation.

2.4. Formulation optimization

In the preliminary experiments, a number of influence factors, such as kinds of polymer material, amount of drug, rotary evaporation temperature, hydration time and volume, were investigated to identify the control factors and their levels during assembly process with EE% and DL% as index. According to the influence degree of each factor, the amount of drug, P123 and Solutol HS15 as three key factors were chosen to follow a CCD [22] to obtain the optimized formulation.

2.5. Characterization of apigenin-loaded polymeric micelles

The morphology of apigenin-loaded polymeric micelles obtained from the optimized formulation was evaluated using a transmission electron microscopy (TEM). Samples were dripped onto copper coated with a carbon grid, then stained by 1 drop of 2% (w/v) phosphotungstic acid, and the excess was wiped off by filter paper. Ultimately, the samples were examined under TEM and photographed after drying [23].

Particle sizes and Zeta potential of the micelles were measured by a Delsa™ Nano C Particle Analyzer (Beckman Coulter. Inc.USA).

The effect of dilution on the stability of micelles was studied based on the previous report [15]. Briefly, the mixed micelle samples were diluted with pH 7.4 phosphate buffer solution (PBS) by grads'multiple and the sizes of the diluted micelles were measured by a Delsa™ Nano C Particle Analyzer (Beckman Coulter. Inc., USA) with Pluronic P123 micelle (containing the same concentration of P 123 and no Solutol HS15) as control.

All measurements were carried out in triplicate.

2.6. Critical micelle concentration (CMC)

The critical micelle concentration (CMC) was determined using pyrene as a fluorescence probe [24]. The concentration of pyrene was fixed at 2.0×10^{-6} mol/L while concentrations of blank micelles were varied from 1×10^{-6} to 4×10^{-4} mol/L. The fluorescence spectra were recorded using a Fluorescence Spectrophotometer with an excitation wavelength of 334 nm. The emission fluorescences at 372 nm and 383 nm were monitored and the CMC was determined at the crosspoint where the intensity ratio I_{372}/I_{383} changed dramatically.

2.7. In vitro drug release study

In vitro drug release profile of apigenin from polymeric micelles was studied with the dialysis method. *In vitro* drug release study was performed with PBS containing 20% (v/v) ethanol as release medium to obtain the sink condition. 600 μL of apigenin-loaded polymeric micelles (amount of apigenin was 192.5 μg) were placed in a dialysis bags (molecular weight cutoff of 1KDa) suspended in 40 mL of release medium at 37 °C, shaking at 100 rpm using an incubating shaker. At the predetermined time intervals, 4 mL of release medium was withdrawn and the same volume of fresh medium was added to maintain the constant volume. The amount of released apigenin was determined at a wavelength of 323 nm using UV–vis method. The apigenin propylene glycol solution as control was done in the same way described above.

2.8. Cell toxicity assays

Cell toxicity was detected by assessing metabolic activity of HepG2 and MCF-7 cells treated with the complexes. HepG2 and MCF-7 cells were obtained from the Institute of Biochemical and Biotechnological Drugs of Shandong University and grown in DMEM and RPMI 1640, respectively. All media were supplemented with 10% FBS, which was named complete medium. Twenty-four hour prior to the assay, cells were seeded on 96-well plates at a density of 5000 cells/well in 200 μL of complete medium. On the day of the assay, the culture medium was replaced with 200 μL of complete medium containing either apigenin DMSO solution or apigenin polymeric micelles with the concentration gradient of apigenin at 32, 16, 8, 4, 2 μg/mL. Following 48 h incubation, the cells were received 200 μL of complete medium and 20 μL of a 5 mg/mL MTT solution after washing with 200 μL of PBS and incubated at 37 °C for 4 h. Then, the medium containing MTT was replaced with 200 μL of DMSO. Finally, the samples were shaking in 96-well plates and the absorbance was measured at 570 nm.

3. Results and discussion

3.1. The optimization of micelle formulation

The CCD is often applied to develop the response surface methodology aiming to optimize formulations with few of factors n ($2 \leq n \leq 6$), and it requires fewer experimental runs than factorial designs, providing savings on time and resources [25]. Before using the design, an overall influence factors during assembly process were investigated to distinguish the impact degree of each factor on the DL%. As a result of the preliminary experiments, the micelles formed by P123 had a higher DL% (0.30%) than those of F127, F68 and PEG-PLA (0.14%, 0.13%, 0.14%, respectively), confirming the superiority in solubilizing the poorly water soluble drugs of P123 as described in the introduction. In addition, amount of drug or Solutol HS15 has an obvious impact on DL% of polymeric micelles. Conversely, the influence of rotary evaporation temperature, hydration time and volume was minor. Therefore, amount of drug, P123 and Solutol HS15 were selected as control factors to perform a CCD. The effect of two variables (amount of apigenin as X_1 in the range from 0.5 mg to 1.68 mg, copolymer carriers composed of P123 and Solutol HS15 in several proportions as X_2 in the range from 0.91 to 15.00) was studied using the CCD at five experimental

Table 1
Central composite design (CCD) of factors with coded values and observed results.

Coded level of variables				True amount (mg)			EE%	DL%
NO.	X1	X2	X3	Apigenin	P123	Solutol HS15		
1	−1	−1	−1	0.83	92.26	29.02	101.2	0.69
2	1	−1	−1	1.68	92.26	29.02	95.3	1.3
3	−1	1	−1	0.83	207.74	29.02	100.2	0.35
4	1	1	−1	1.68	207.74	29.02	98.42	0.7
5	−1	−1	1	0.83	92.26	80.98	92.23	0.43
6	1	−1	1	1.68	92.26	80.98	100.8	0.97
7	−1	1	1	0.83	207.74	80.98	100.2	0.28
8	1	1	1	1.68	207.74	80.98	100.4	0.58
9	−1.73	0	0	0.5	150	55	99.5	0.24
10	1.732	0	0	2	150	55	97.82	0.95
11	0	−1.732	0	1.25	50	55	99.45	1.17
12	0	1.732	0	1.25	250	55	86.63	0.35
13	0	0	−1.732	1.25	150	10	95.95	0.74
14	0	0	1.732	1.25	150	100	97.39	0.49
15	0	0	0	1.25	150	55	96.81	0.59

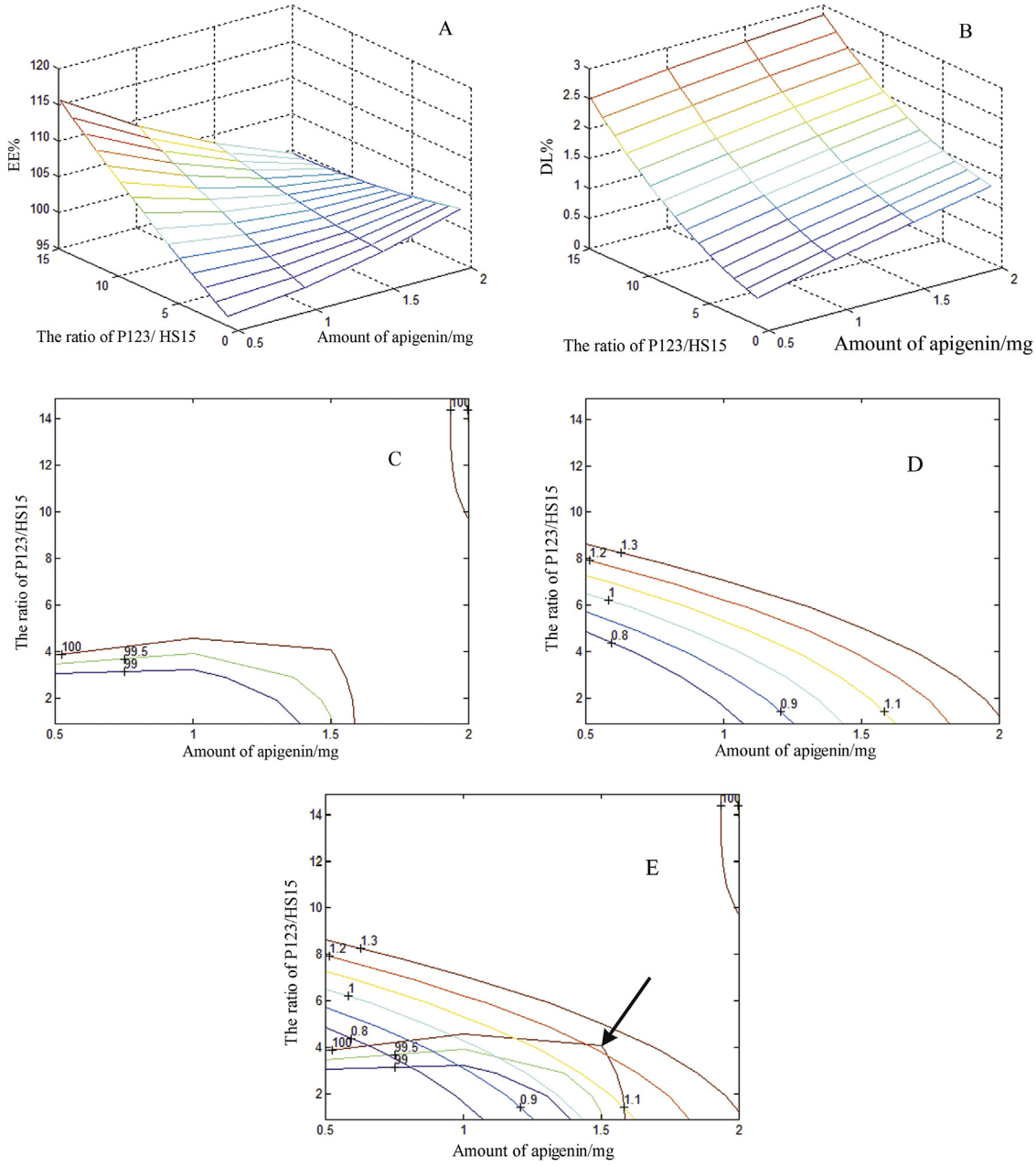

Fig. 1. Predicted response surface plots and response surface contour plots. Response surface plots of EE% (A) and DL% (B), the response surface contour plots of EE% (C) and DL%(D), and the mixed response surface contour plot of EE% and DL% (E). The common part shown in (E) was the best chosen area for the amount of apigenin and the ratio of P123/HS15.

levels [26], 15 required experiments and the combinations as well as the results were shown in Table 1. SPSS 17.0 was employed to deal with the data to get regression equations. The quadratic polynomial equations were as follows:

$$Y_{EE\%} = 2.041X_1^2 - 1.069X_1X_2 + 1.711X_2 + 0.013X_2^2 + 94.76$$

$$YDL\% = 0.689X_1 - 0.046X_1^2 - 0.063X_2 - 0.023X_1X_2 + 0.006X_2^2 + 0.075$$

where X_1 represents the amount of apigenin, X_2 represents ratio of P123/HS15. From the formula, all the dependent variables can be well described by quadratic polynomial with high correlation coefficient ($R^2 > 0.90$, $P < 0.005$).

Response surface plots (Fig. 1A and B) were described according to regression equations with Matlab (R2010b). The estimated response surface contour plots were designed to search an optimal result (Fig. 1C and D) and the common part of the contour plot between EE% and DL% was the best chosen area for the amount of apigenin and the ratio of P123/HS15. Results are shown in Fig. 1E.

From the results of EE% and DL% in Table 1, it can be concluded that each factor has a significant impact on DL% while very slight on EE%, which means that DL% plays a key role for the selection of formulation compositions under the condition that values of EE% was generally high. Therefore, taking higher DL% as index, the optimum formulation was selected as follows: apigenin 1.68 mg, P123 92 mg, Solutol HS15 29 mg. And the average EE% and DL% of the optimal formulation were 96.36% and 1.32%, respectively. Furthermore, the concentration of apigenin in the obtained apigenin-loaded

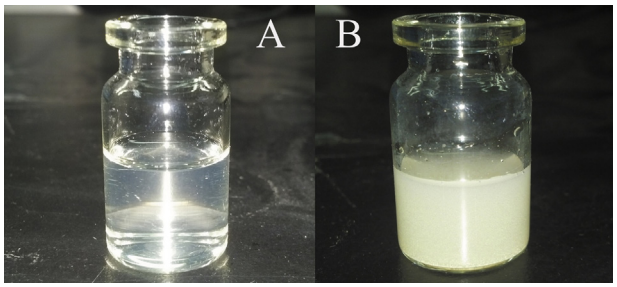

Fig. 2. Photographic images of apigenin-loaded micelles (A) and apigenin suspension (B). The obtained drug-loaded micellar solution was clear and homogeneous as shown in (A), with the suspension of crude apigenin dispersed in water at the same drug concentration as control (B).

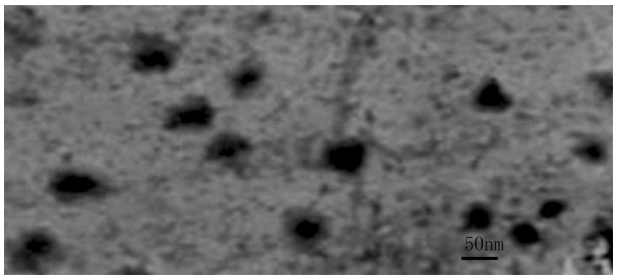

Fig. 3. The morphology of apigenin-loaded polymeric micelles obtained from the optimized formulation (×10,000).

micellar solution was about 320.8 μg/mL, which significantly increased the solubility of apigenin in water (2.16 μg/mL). In other words, the solubility of apigenin in the mixed micelles was about 148-fold that of crude apigenin in water.

3.2. Characterization of apigenin-loaded polymeric micelles

The obtained apigenin-loaded micelles was clear and homogeneous as shown in Fig. 2(A), with the suspension of crude apigenin dispersed in water at the same drug concentration as control Fig. 2(B). The morphology of the optimal formulation under transmission electron microscope (TEM) presented homogeneous spheres as photographed in Fig. 3. The average diameter of micelles and the Zeta potential was 16.9 nm with the polydispersity of 0.046 (Fig. 4A) and −5.87 mV (Fig. 4B), respectively, as measured with a Delsa™ Nano C Particle Analyzer. Homogeneous and small particle sizes (<200 nm) could reduce the uptake of the reticuloendothelial system (RES) and provide efficiently passive tumor-targeting ability *via* the EPR effects [27]. Hydrogen bond may occur between the phenolic hydroxyl group of apigenin and the carboxyl of the PEG chains in apigenin loaded micelles, leading to a slightly decreased particle size compared with that of the blank micelle (18.9 nm) [28]. The negative potential of the micelles might be related to the formed hydrogen bonds between the phenolic hydroxyl groups of apigenin associated in the corona or shell of micelles and the corresponding groups such as hydroxyl groups in the PEG chains of Solutol HS 15 and PEO chains of P123 [29,30].

The results of the effect of dilution on the stability of micelles were showed in Table 2. Obviously, the particle size of the

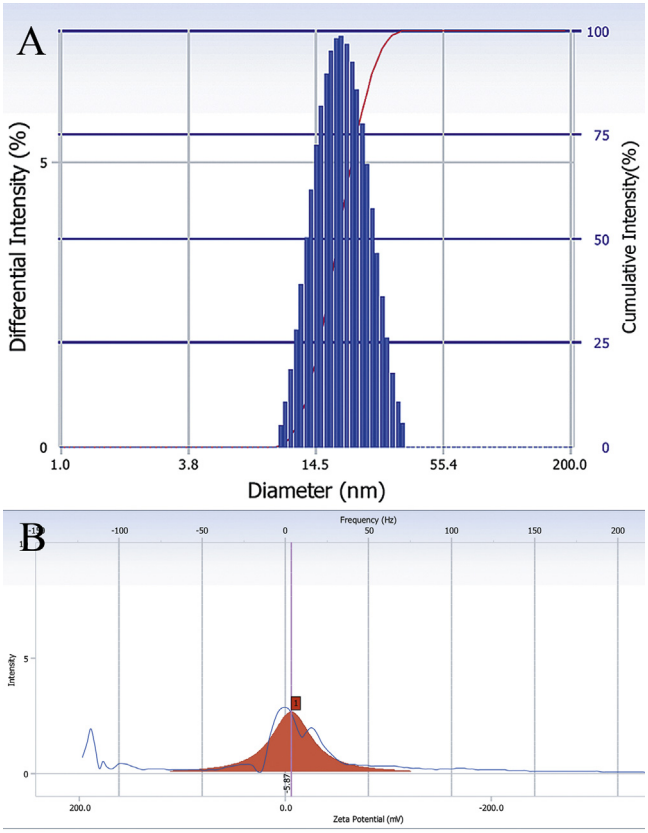

Fig. 4. The size (A) and Zeta potential (B) of the apigenin-loaded polymeric micelles.

mixed micelle showed a relatively stable property against dilution whereas that of the P123 micelle varied significantly in a large range. What's more, the particle size of the P123 micelle was unfit for measure when diluted 100 times, the reason might be that an extra-low particle concentration was beyond the detection limit of Delsa™ Nano C Particle Analyzer, or the structure of P123 micelle was dissociated. Because the shell of P123 micelle formed by the weak interaction between PEO–PPO–PEO monomer was thin and prefered to be attacked when diluted, and then the micelle particle became more loosely organized, leading to an increasing particle size and dissociation tendency. In contrast, the incrassated corona or shell of the mixed micelle formed by the PEG chains of Solutol HS 15 and hydrophilic PEO part in PEO–PPO–PEO led to a hydrated shell wrapping outside, which was a stronger protective obstacle for the core of the micelle.

3.3. Critical Micelle Concentration (CMC)

Pyrene is a kind of important fluorescence probe with long fluorescent lifetime, enough sensitivity to the change of polarity. When the concentration of pyrene is less than 10^{-5} mol/L, monomer excitation fluorescence with fine structure can be detected and five-line peaks are on the spectrum of emission fluorescence of pyrene probe with a wavelength of 372, 379, 383, 390 and 480 nm, nearly. Intensity ratio of the first and third peak (I_{372}/I_{383}) can be transformed

Table 2
Variation of particle size against dilution with pH 7.4 PBS.

Micelle	Diluted Times					
	0	5	10	20	50	100
Mixed micelle	17 nm	17.6 nm	17.9 nm	18.6 nm	18.8 nm	19.3 nm
P123 micelle	21.1 nm	27.2 nm	66.3 nm	86.2 nm	97.4 nm	–

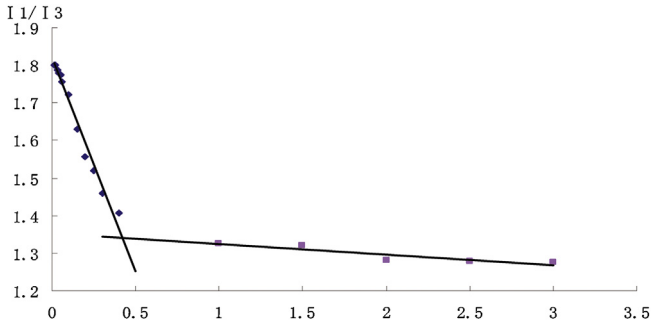

Fig. 5. Plot of I_{372}/I_{383} *versus* concentrations of copolymers in distilled water.

with the change of polarity around. Pyrene is hydrophobic with poor solubility in water but has an increased solubility in micelles. When the concentration of polymer is less than CMC, the rate of I_{372}/I_{383} changes slightly, while it increases dramatically when the concentration of polymer reaches the CMC. The phenomenon is related to the fact that pyrene can move into the inside of the micelles from the aqueous phase once the micelle forms, which results in an alteration in the polarity around. Consequently, CMC could be calculated with the point where the I_{372}/I_{383} increased dramatically (Fig. 5).

The CMC value of the micelle was as low as 4.23×10^{-5} mol/L, which was in accordance with previous report (CMC for P123 was 1.97×10^{-5} mol/L) [25]. The presence of Solutol HS15 did not result in notable variation in CMC of P123. The low CMC of the copolymers suggested that the micelle composed of P123 and Solutol HS15 should have good stability and be able to maintain the integrity of micelles toward dilution.

3.4. In vitro drug release study

In vitro drug release of apigenin from polymeric micelles was studied by dialysis with PBS solution containing 20% (v/v) ethanol at 37 °C as release medium to obtain the sink condition. The change of release rate of apigenin with time was shown in Fig. 6. Apparently, apigenin released from polymeric micelles was much slower in comparison to that of the propylene glycol solution. In addition, the drug release from micelles started with an initial burst in the first hour, followed by a stably sustained release behavior,

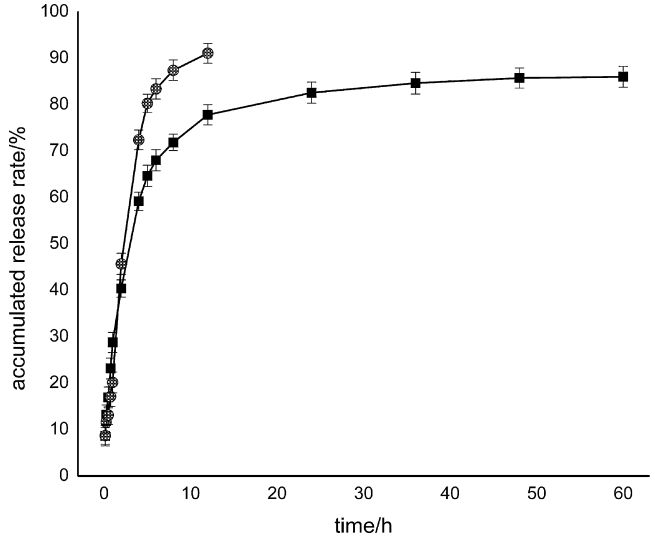

Fig. 6. *In vitro* release profiles of apigenin-loaded polymeric micelles (■) and apigenin propylene glycol solution (●).

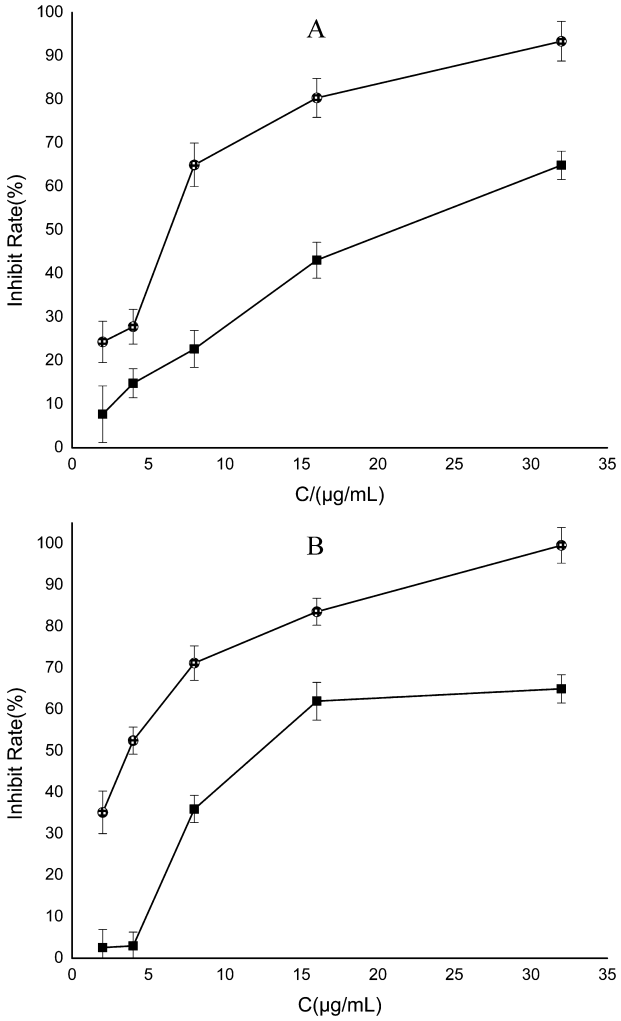

Fig. 7. The effects of apigenin-loaded polymeric micelles (●), apigenin DMSO solution (■) on inhibit rate of Hep G2 (A) and MCF-7cells (B).

which was in coincidence with the distribution of drug in the mixed micelles as shown in Graphical Abstract

The initial burst might be related to the fact that a small quantity of the drug associated in the hydrophilic corona or shell of micelles could proceed by double effects *via* both the hydration of the interfacial drug molecules and their passive diffusion [15]. However, the drug incorporated into the inner core of micelle released by drug diffusion and the erosion or swelling of polymer materials [31], leading to a stably sustained release in the following phase.

3.5. Cell toxicity assays

Cytotoxicity of the polymeric micelles was investigated with MTT assay which evaluated metabolic activity of cells treated with the complexes. It demonstrated that the general trend between inhibition rate and the concentration of apigenin was the same in both HepG2 and MCF-7 cells. The inhibition rates of apigenin-loaded polymeric micelles on both HepG2 (Fig. 7A) and MCF-7 (Fig. 7B) were higher than that of apigenin DMSO solution, which could be explained by the enhanced solubility of the poorly soluble apigenin in micelle solution, and better uptake of apigenin-loaded micelles by the cells. In addition, the sensitivity of MCF-7 to apigenin was higher compared to that of HepG2, which might be attributed to different mechanism of apigenin-induced cell death in the two cell lines. As explained in previous literature, apigenin could induce the growth inhibition of HepG2 cell by the

method of increasing the release of cytochrome C from mitochondrion to the cytoplasm, causing a DNA fragmentation in HepG2 cells [32]. Whereas different from HepG2, MCF-7 can express two key cell cycle regulators, wild-type p53 and the retinoblastoma tumor suppressor protein (Rb). Apigenin can induce a significant decrease in cyclin B1 and CDK1 protein levels, resulting in a marked inhibition of CDK1 kinase activity. In addition, the prevention of the phosphorylation of Rb leads to a cell apoptosis in G2/M phase after apigenin treatment [33]. The IC50 values on HepG2 cells for apigenin-loaded polymeric micelles and free apigenin in DMSO solution were 5.57 μg/mL, 20.19 μg/mL, respectively, while 3.75 μg/mL, 16.62 μg/mL for MCF-7 cells. As a result, it can be concluded that the MCF-7 cells can demonstrate considerably higher response to the cytotoxic effect of the apigenin compared to HepG2 cells.

4. Conclusion

In the present study, apigenin-loaded micelles were fabricated by a thin-film dispersion method. The mixed polymeric micelles, composed of P123 and Solutol HS15, exhibited higher EE% and DL% for apigenin. The average size and the Zeta potential of the apigenin-loaded mixed micelles were 16.9 nm and −5.87 mV, respectively. The low CMC (4.23×10^{-5} mol/L) of the micelles meaned that they could keep a stable micelle structure even in a diluted environment. The *in vitro* release behavior of apigenin from the micelles showed the preparation possessed a sustained-release property. While the results from *in vitro* cytotoxicity studies on HepG2 and MCF-7 cells demonstrated that apigenin-loaded micelles were effective in inhibiting the growth of tumor cells. The quantitative and qualitative results provided the evidence that polymeric micelle formulation developed in this study can become a potential nanocarrier for apigenin as a poorly water soluble drug.

Acknowledgement

This work was partly supported by the Natural Science Foundation of Shandong Province, China (no. ZR2011 HM026).

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