International Journal of Pharmaceutics 369 (2009) 19–23

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Tyrosine kinase inhibitor loaded PCL microspheres prepared by S/O/W technique using ethanol as pretreatment agent

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ARTICLE INFO

Article history:
Received 16 May 2008
Received in revised form
22 September 2008
Accepted 20 October 2008
Available online 7 November 2008

Keywords:
Tyrosine kinase inhibitor
Poly(ε-caprolactone)
Microspheres
S/O/W

ABSTRACT

A new tyrosine kinase inhibitor (denoted as CH331) and its poly(ε-caprolactone) (PCL) microspheres were studied. The CH331 particles were pretreated with ethanol and then used to prepare CH331 loaded PCL microspheres by S/O/W solvent evaporation technique. Solubility values of CH331 in several organic and aqueous media were measured. The amount of ethanol and CH331 solubility play a significant role in drug loading, encapsulation efficiency, mean diameter and morphology of the microspheres, crystallinity and in vitro drug release. The treatment with a suitable amount of ethanol leads to more uniform sizes, better appearance and higher encapsulation efficiency for the microspheres. Compared with 0.5% PVA phosphate buffer solution (pH 7.4), 0.5% PVA aqueous solution as outer aqueous phase lowers encapsulation efficiency of microspheres, however, improves the drug release behavior.

1. Introduction

A new tyrosine kinase inhibitor (denoted as CH331, Fig. 1), one of quinazoline compounds, has been recently synthesized by the Cancer Center of Sun Yat-sen University (Guangzhou, China). It is a novel inhibitor of vascular epidermal growth factor (VEGF) receptor tyrosine kinase (TK) with improved specificity, selectivity and clinical efficacy for tumor therapy (Sun et al., 2007). TK, which is activated by binding with VEGF, plays an important role in the biochemical signal transduction across cytoplasmic membrane, and can influence growth and metastasis of tumor. CH331 can inhibit tyrosine kinase activity, thereby blocking VEGF-induced angiogenesis. As CH331 is a novel potential antitumor agent, data is lacking on its physical and chemical character. To date, there is no investigation on the microencapsulation of CH331 reported as author knows.

Selection of microencapsulation technique is primarily determined by the solubility of the drug and the polymer in various systems (Nihant et al., 1994). Solvent evaporation technique is one of the most popular ways to prepare microspheres (Pekarek et al., 1996; Youan et al., 1999; Shi et al., 2003; Zhang and Zhu, 2004; Wang et al., 2005). S/O/W method is commonly applied for the encapsulation of water-soluble drugs into microparticles (Cleland and Jones, 1996; Takada et al., 1997; Lamprecht et al., 2000; Castellanos et al., 2001). Hydrophilic additives, such as water, ethanol and glycerol, were added into dichloromethne containing crystalline insulin so as to suppress initial burst release of insulin (Yamaguchi et al., 2002).

Poly(ε-caprolactone) (PCL) is a biocompatible and biodegradable polyester that has been investigated for controlled delivery of several low molecular weight drugs (Pitt, 1990). Other advantages of PCL include hydrophobicity, in vitro stability and low cost. Therefore, many investigations have focused on drug-loaded PCL microspheres in recent years (Lin and Huang, 2001; Gibaud et al., 2004; Kim et al., 2005; Wang et al., 2008).

In this study, we first measured the solubility values of CH331 in some organic and aqueous media, then encapsulated CH331 into PCL microspheres by S/O/W solvent evaporation technique using ethanol as drug particle pretreatment agent, and investigated the effect of the amount of ethanol and the outer aqueous phase on CH331 loaded PCL microspheres.

2. Materials and methods

2.1. Materials

Tyrosine kinase inhibitor (CH331) was a gift offered by the Cancer Center of Sun Yat-sen University (Guangzhou, China). Poly(ε-caprolactone) (PCL) (MW 50,000) was supplied by Daicel Polymer Ltd. (Minatoku, Tokyo, Japan). Polyvinylalcohol (PVA,

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doi:10.1016/j.ijpharm.2008.10.032

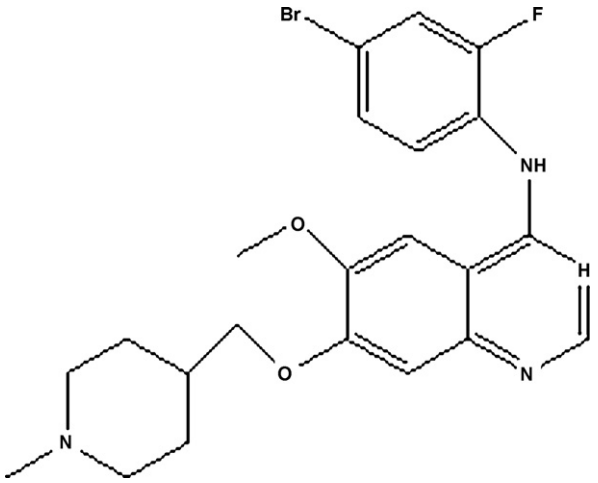

Fig. 1. Chemical structure of tyrosine kinase inhibitor.

88% hydrolyzed) was provided by Weicheng Chemical Industry Co. Ltd. (Shanghai, China) as emulsifier. Hydrochloric acid was purchased from Lingfeng Chemical Reagent Co. Ltd. (Shanghai, China). Dichloromethane and ethanol were obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

2.2. Measurement of CH331 Solubility

The solubility of CH331 was measured in distilled water, pH 6.8 and 7.4 phosphate buffered solutions (PBS), pH 1.2 hydrochloric acid (pH 1.2 HCl), ethanol, dichloromethane and chloroform, respectively.

Excess amount of CH331 was added to 3 ml of solvent above in a 5 ml centrifuge tube and shaken in a water bath at 37 ± 0.5 °C for 48 h to achieve dissolution equilibrium, then samples were centrifuged for 10 min at 12,000 rpm to separate undissolved CH331. The supernatant was diluted and assayed spectrophotometrically (Spectrumlab54 UV spectrophotometer, Lengguang technology Co. Ltd., Shanghai, China) at its maximum absorption wavelength in the relevant medium.

2.3. Preparation of microspheres

CH331 loaded PCL (CH331-PCL) microspheres were prepared by a modified S/O/W solvent evaporation method. First, 1000 mg of PCL polymer was dissolved in 4 ml of dichloromethane, then 200 mg of CH331 was dispersed in different amounts of ethanol (1, 2 or 2.5 ml), homogenized for 60 s by Ultrasonic Cell Disruptor (Soniprep 150, Sanyo, England), and added into polymer solution. This S/O dispersion was ultrasonicated for another 60 s and added dropwise into 100 ml of 0.5% (w/v) PVA outer phase (H_2O or pH 7.4 PBS). The resulting emulsion was stirred with a double-bladed propeller at 25 °C for 1 h under ambient pressure, and another 30 min under reduced pressure (20 Pa), while the stirring rate was 1000 rpm. The microspheres were collected by filtration in vacuum, washed three times with 20 ml distilled water each, and dried in a vacuum desiccator at room temperature.

2.4. Measurement of drug loading

20 mg of CH331-PCL microspheres were dissolved in 0.5 ml of dichloromethane in a 5 ml centrifuge tube, and then 3 ml of pH 1.2 HCl was added and vortexed for 5 min. The resulting solution was centrifuged for 5 min at 12,000 rpm. Supernatant was diluted properly in pH 1.2 HCl to measure CH331 concentration at the maximum wavelength of 251 nm by UV spectrophotometry. The calibration curve equation used for calculating the concentration of CH331 is $A = 0.0384C + 0.0065$ ($r^2 = 0.999$).

The drug loading and entrapment efficiency were calculated as follows:

$$\text{drug loading}\,(\%) = \frac{\text{mass of drug in microspheres}}{\text{mass of microspheres}} \times 100 \quad (1)$$

$$\text{encapsulation efficiency}\,(\%) = \frac{\text{drug loading}}{\text{theoretical drug loading}} \times 100 \quad (2)$$

2.5. Analysis of particle size

Microspheres sizes were analysed by a particle micrograph analyzer (Winner 99, WXO6301, Winner Science and Technology Co. Ltd., JiNan, China). For the analysis, the sample was put onto a slide and calculated its particle distribution using a computer program supplied by the manufacturer. Then the mean particle size was calculated through the equation as follows:

$$\bar{d} = \frac{\left(\sum_{i=1}^{500} n_i d_i\right)}{\sum_{i=1}^{500} n_i} \quad (3)$$

where d_i is the particle size of the microspheres, and n_i is the number of microspheres whose particle sizes are d_i.

2.6. Morphology of microspheres

The shapes and surface characteristics of microspheres were investigated and photographed using scanning electron microscope (SEM) (JSM-7401F, JEOL, Japan). Dried microspheres were mounted onto brass stubs using double-sided adhesive tape with conductive effect and analyzed with SEM.

2.7. X-ray diffraction

X-ray diffraction patterns were recorded with an X-ray diffractometer (D/max 2200, Rigaku, Japan). The samples were placed in a steel holder and exposed over a 2θ range of 0–40° at the rate of 6° min^{-1}. The degree of crystallinity (X_C%) was evaluated from the following equation (Gonzalez et al., 1999):

$$X_C\ (\%) = \frac{I_T - I_A}{I_T} \times 100 \quad (4)$$

where I_T and I_A are the intensities of the whole sample and the amorphous phase (area under the curve), respectively.

2.8. In vitro drug release

A determined amount of CH331-PCL microspheres in 10 ml of pH 7.4 PBS was shaken in a 37 ± 0.5 °C water bath at 110 rpm. At predetermined time intervals, samples were centrifuged for 5 min at 4000 rpm, then 4 ml of supernatant was withdrawn and 4 ml of fresh dissolution medium was added. The supernatant was filtrated through 0.45 μm Millipore filter, diluted and analysed spectrophotometrically at 247 nm.

3. Results and discussion

3.1. Solubility of CH331

CH331 is a new compound, which can be used as a tyrosine kinase inhibitor. It is necessary to study its solubility in organic and aqueous media for effective encapsulation of CH331.

Table 1
Solubility data of CH331 at 37 °C ($n = 3$).

Solvent	Solubility (mg/ml)	R.S.D. (%)
H_2O	52.74 ± 0.89	1.86
pH 6.8 PBS	13.06 ± 0.21	1.58
pH 7.4 PBS	3.64 ± 0.06	1.67
pH 1.2 HCl	51.06 ± 0.25	0.49
Ethanol	13.66 ± 0.23	1.68
Dichloromethane	0.81 ± 0.01	1.23
Chloroform	3.14 ± 0.05	1.59

As shown in Table 1, compared with organic solvents (chloroform, dichloromethane and ethanol), CH331 has a higher solubility in water. The solubility of CH331 decreases with the increasing pH in aqueous media. CH331 is soluble in ethanol to a certain extent, and poorly soluble in dichloromethane. Solubility of CH331 relates to the structure of CH331 molecule (Fig. 1), which is one of quinazoline compounds, an organic weak base.

3.2. Effect of ethanol on S/O dispersions

Based on the solubility in organic and aqueous media of CH331, we tried to prepare CH331 loaded PCL microspheres by S/O/W solvent evaporation technique. Dichloromethane was used as inner oil phase, and aqueous media as outer phase. The mean size of CH331 particles used is about 10 μm. The drug particles would congregate into bigger granules when they were added into dichloromethane, even if a homogenizer was used (Fig. 2a). Moreover, fluidity of the dispersion was not very well. It is difficult to form homogeneous microspheres due to drug aggregation and poor fluidity in inner oil phase. Different amounts of ethanol ranged from 0.5 to 2.5 ml were investigated to pretreat CH331 particles. 200 mg of drug particles was first treated with a certain amount of ethanol, and then added into 4 ml of dichloromethane containing 1000 mg PCL. As shown in Fig. 2b, there was no fluidity when 0.5 ml of ethanol was used; 1 and 2 ml of ethanol led to good fluidity and transparency of S/O dispersions. However, when 2.5 ml of ethanol was used, a little precipitates occurred in S/O dispersion. This is because ethanol is a poor solvent for PCL, some PCL will precipitate in organic phase as the amount of ethanol increases. Pretreatment with a few amount of ethanol can partly dissolve CH331 particles to make them smaller and disperse homogeneously in dichloromethane, and also prevent CH331 particle aggregation in inner oil phase.

3.3. Encapsulation efficiency and particle size

As mentioned above, when CH331 particles were not pretreated with ethanol, the drug particles gathered into a mass in organic phase, which made it difficult to prepare microspheres. If the amount of ethanol treated was not enough, such as 0.5 ml, we also could not prepare drug loaded microspheres. It was found that uniform microspheres could be prepared after pretreatment of the drug particles with 1–2.5 ml of ethanol.

As listed in Table 2, the increasing amount of ethanol leads to a decrease in encapsulation efficiency or drug loading when the outer phase is 0.5% PVA–PBS (pH 7.4). When the amount of ethanol is the same (1 ml), compared with 0.5% PVA–PBS (pH 7.4), 0.5% PVA–H_2O as outer aqueous phase causes much lower encapsulation efficiency or drug loading, implying that outer aqueous phase plays a significant role in the encapsulation of CH331. It might be due to the difference of drug solubility in outer aqueous phases. The solubility values of CH331 in pH 7.4 PBS and H_2O are 3.64 and 52.74 mg/ml, respectively. Higher solubility of CH331 in outer phase may result in more partition of drug into outer aqueous phase, thus decreasing encapsulation efficiency and drug loading.

The particle size of microspheres decreases with the increasing amount of ethanol, while the change of outer aqueous phase exerts almost no influence on it (Table 2). Moreover, the increasing amount of ethanol also increases inner oil phase volume. It was observed from our experiment that the viscosity of oil phase decreased (fluidity increased) and the clarity increased with the increasing amount of ethanol, which led to smaller emulsion droplets. Size of microspheres is commonly determined by the size of emulsion droplets formed in the preparation process (Sansdrap and Moës, 1993; Wang and Guo, 2008).

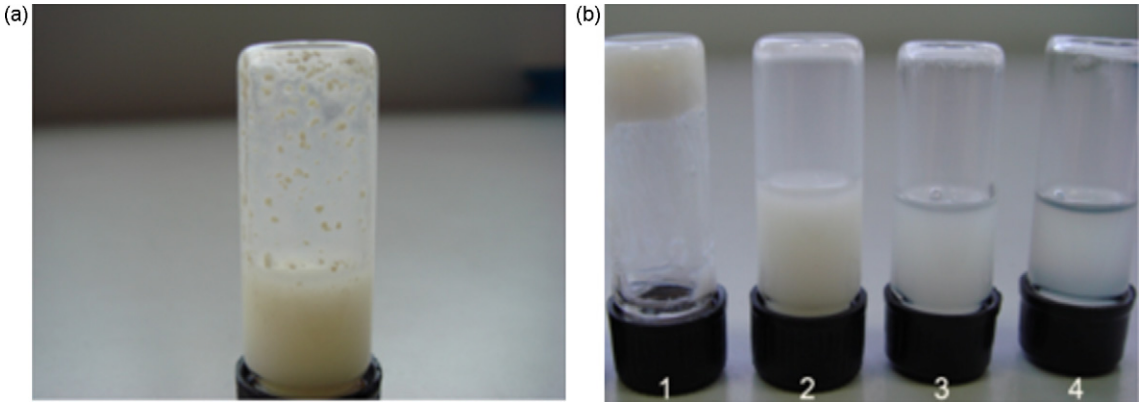

Fig. 2. Photographs of S/O dispersion with different amounts of ethanol: (a) no ethanol; (b_1) 0.5; (b_2) 1; (b_3) 2; (b_4) 2.5 ml.

Table 2
Drug loading, encapsulation efficiency and particle size of CH331-PCL microspheres obtained under various preparation conditions ($n = 3$).

Batch	Ethanol (ml)	Outer phase	D.L. (% ± S.D.)	E.E. (% ± S.D.)	Particle size (μm)
B1	1	0.5% PVA–PBS (7.4)	11.64 ± 0.10	58.20 ± 0.51	94.80 ± 19.09
B2	2	0.5% PVA–PBS (7.4)	10.21 ± 0.17	51.03 ± 0.85	76.86 ± 16.09
B3	2.5	0.5% PVA–PBS (7.4)	6.98 ± 0.05	34.88 ± 0.24	58.40 ± 15.93
B4	1	0.5% PVA–H_2O	4.18 ± 0.09	20.92 ± 0.43	90.17 ± 18.68

D.L., drug loading of the microspheres; E.E., encapsulation efficiency of CH331.

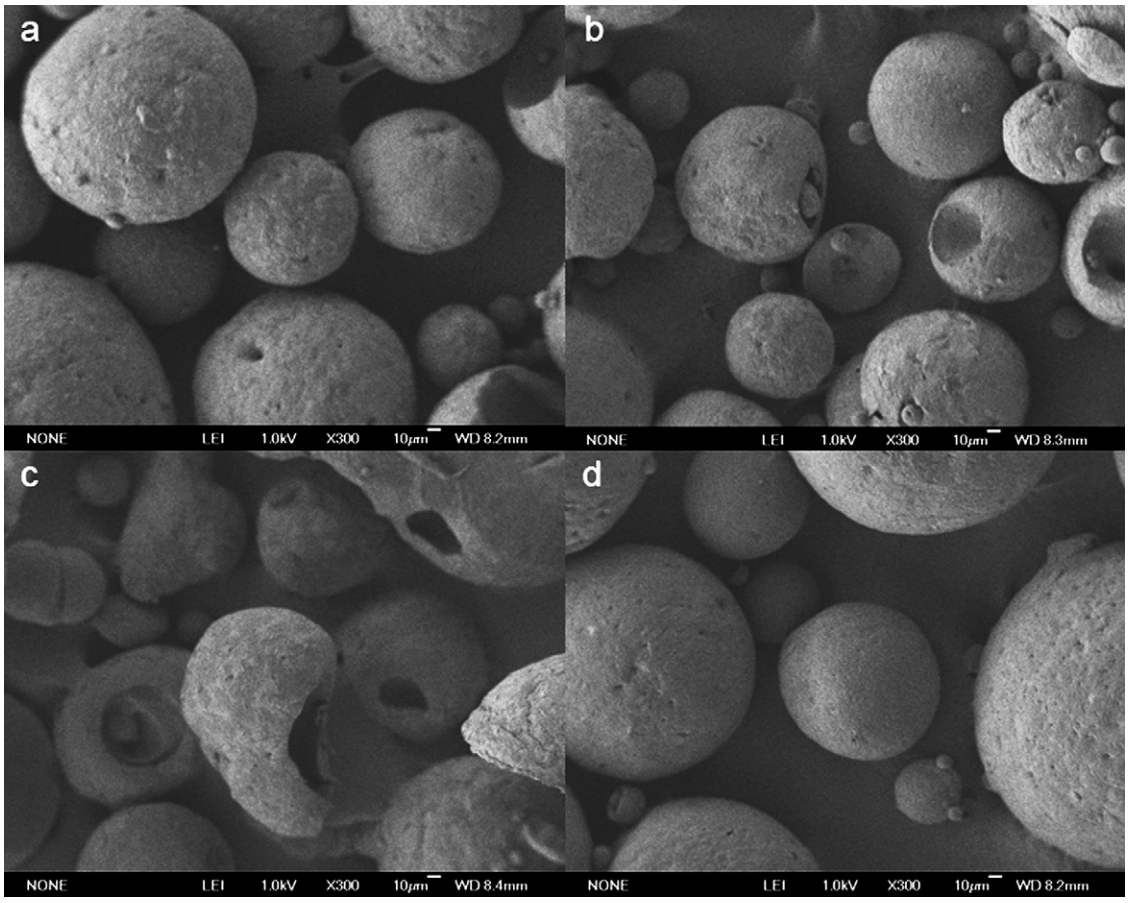

Fig. 3. Scanning electron micrographs of the microspheres B1 (a), B2 (b), B3 (c) and B4 (d).

3.4. Morphology of microspheres

The amount of ethanol plays a significant role in the morphology of microspheres and influences roundness and roughness of microspheres. As shown in Fig. 3, the roundness and smoothness of microspheres become worse as the amount of ethanol increases. B1 and B2 microspheres are spherical, but there are more pits on the surface of B2 microspheres; B3 microspheres become irregular and have the most pits on the surface; B4 microspheres are spherical and smooth, there is no pit on the surface. PCL is almost insoluble in ethanol, the higher amount of ethanol leads to higher proportion of ethanol in inner oil phase and then decreases PCL solubility in it, which makes the morphology of microspheres become worse.

3.5. X-ray diffraction analysis

CH331, blank PCL microspheres and CH331-PCL microspheres were characterized using X-ray diffraction. As shown in Fig. 4a, the pattern of blank PCL microspheres displays two characteristic peaks in the range of 20–25°, confirming semi-crystalline structure of PCL polymer (Pérez et al., 2000). The characteristic peak of CH331 (Fig. 4b) does not occur in the diffraction patterns of CH331-PCL microspheres (Fig. 4c–f), which indicates that CH331 was dissolved or amorphous in microspheres (Pérez et al., 2000; Jeong et al., 2003). The result is probably due to the fact that the crystallinity of CH331 is destroyed after treatment with ethanol.

Blank PCL microspheres have higher crystallinity than that of drug-loaded microspheres (Table 3). This might be due to the disturbance of drug molecules to PCL crystallization.

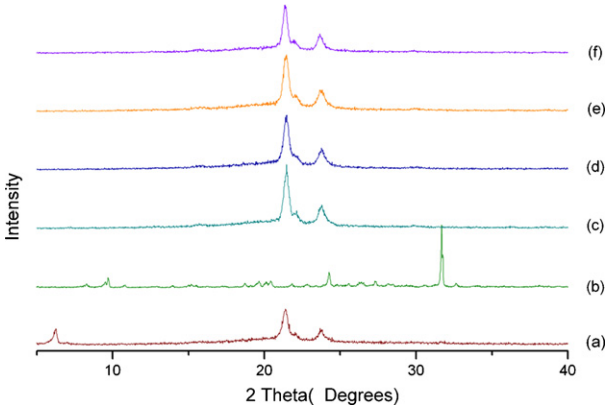

Fig. 4. X-ray diffraction patterns of: (a) blank PCL microspheres; (b) CH331; (c–f) microspheres B1–B4.

Table 3
PCL crystallinity in microspheres (the amount of PCL was fixed at 1000 mg).

Sample	Crystallinity (%)
Blank PCL microspheres	45.30
B1	37.99
B2	39.84
B3	39.58
B4	35.78

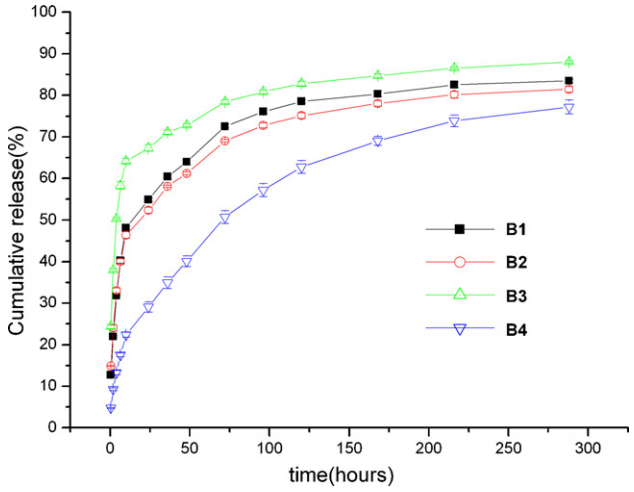

Fig. 5. In vitro release profiles of CH331 from the microspheres in phosphate buffer (pH 7.4) at 37 °C ($n = 3$).

3.6. In vitro drug release

In vitro release profiles of CH331 from the microspheres in pH 7.4 PBS (Fig. 5) exhibit a rapid release phase followed by a slow release phase. During the rapid release phase, the release rate of B4 is also lower. The initial 0.5 h release for B1 to B3 were 12.74%, 14.90%, 24.37%, respectively, while only 4.75% of CH331 from B4 was released, which elucidates higher burst effect for B1 to B3. About 77–88% of the entrapped drug released within 288 h, indicating a certain extent of sustained release.

The result suggests that the drug has lower burst effect and releases slower when the outer phase is 0.5% PVA–H_2O. The probable reason is that high solubility of drug in H_2O might make the drug distributed at the surface of microspheres migrate into the outer phase during the preparation process, thus decreasing the burst effect, for the burst effect is mainly due to release of drug located near the microsphere surface (Jameela et al., 1997; O'Donnell and McGinity, 1997).

4. Conclusion

Solubility values of CH331 in some organic and aqueous media were obtained. On the basis of it, CH331 loaded PCL microspheres were prepared by S/O/W solvent evaporation technique. Pretreatment of CH331 particles with a suitable amount of ethanol is necessary for the preparation of microspheres. The amount of ethanol used has a significant effect on drug loading, encapsulation efficiency, microsphere size and morphology, as well as drug release behavior. Compared with 0.5% PVA–PBS (pH 7.4), 0.5% PVA–H_2O as outer aqueous phase produces microspheres with lower drug loading and an improved drug release behavior.

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