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Preparation and physicochemical characteristics of the complex of edaravone with hydroxypropyl-β-cyclodextrin

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ABSTRACT

The aim of this study was to prepare an inclusion complex of edaravone, a novel free radical scavenger drug, with hydroxypropyl-β-cyclodextrin (HP-β-CD) to improve its apparent solubility, dissolution rate and stability. The solubility of edaravone was evaluated by phase solubility method and the profile displayed a typical A_L-type, indicating the formation of 1:1 stoichiometric inclusion complex. The inclusion complex was prepared by freeze-drying method and characterized by DTA, XRD and NMR. These results suggested that edaravone could form inclusion complex with HP-β-CD and was deeply included in the cavity of HP-β-CD. Additionally, the dissolution rate and stability of the inclusion complex were greatly improved compared with that of edaravone. These results strongly showed that the use of HP-β-CD could be a promising approach to improve the physicochemical characteristics of edaravone.

1. Introduction

Edaravone (EDA, 3-methyl-1-phenyl-2-pyrazolin-5-one, Fig. 1) is a novel free radical scavenger, and was the first neuroprotective agent that was clinically used for the treatment of acute cerebral infarction (Nakamura et al., 2008). By inhibiting the lipid peroxidation, EDA can be used to protect brain cells, vascular endothelial cells and nerve cells from the oxidative damage (Wang, 2004; Sato, Mizuno, & Ishii, 2009). In addition, it has been reported that EDA can improve the regional blood flow after cerebral infarction by attenuating the brain edema (Mitsumori, Sakuragi, Kitami, Koyanagi, & Murata, 1998), and counteract the development of multiple low-dose streptozotocin-induced diabetes in mice (Fukudome, Matsuda, Kawasaki, Ago, & Matsuda, 2008). However, the poor solubility and stability of EDA have largely influenced its oral bioavailability, and the oral formulation of EDA has not yet been used clinically by now. Therefore, considerable attentions have been paid to how to increase the water solubility and physicochemical stability of EDA and to develop its high-quality oral formulation.

Cyclodextrins (CDs) are cyclic oligosaccharides, which have been recognised as useful pharmaceutical excipients (Loftson & Brewster, 1996). CDs can interact with appropriately sized molecules to form the inclusion complexes. These inclusion complexes have been successfully used to improve the solubility, stability and bioavailability of many compounds (Peggy, Maria, & Beatriz, 2010). But due to the problems encountered with the aqueous solubility of CDs, their derivatives with increased aqueous solubility properties have been developed (Duchene & Wouessidjewe, 1990). Hydroxypropyl-β-cyclodextrin (HP-β-CD), a hydroxyalkyl derivative of β-CD, was developed and widely studied in the field of drug encapsulation because of its inclusion ability along with high water solubility (Misiuk & Zalewska, 2009). Besides, toxicological studies have pointed out that HP-β-CD is well tolerated in humans by both oral and intravenous administration (Sarah & Robert, 2005).

So far, several studies have been performed on the reaction between HP-β-CD and some poorly water-soluble or unstable organic compounds (Onyeji, Omoruyi, & Olandimeji, 2007; Wu, Liang, Yuan, Wang, & Yan, 2010; Yan et al., 2008), but the solid inclusion complex of EDA with HP-β-CD has not yet been reported. In this study, we investigated the complexation of EDA with HP-β-CD with the aim to improve the solubility and stability of this drug, and characterized the physicochemical properties of the formed complex. The complex with HP-β-CD was prepared by a freeze-drying method at stoichiometric ratio. The type and the stability constant of the complex were established according to phase solubility studies. Differential thermal analysis (DTA), X-ray diffraction (XRD) and NMR were used to characterize the product. Addition-

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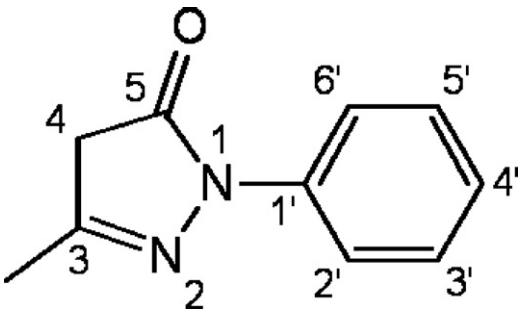

Fig. 1. the structure of EDA.

ally, the dissolution properties of the complex EDA were evaluated and compared with that of free EDA.

2. Materials and methods

2.1. Materials

EDA (purity: 99.62%) and HP-β-CD (DS = 6.0) were purchased from Jinan Zhongke Yitong Chemical Co. Ltd. (Shandong, China) and Taixin Yimin Chemical Co. Ltd. (Jiangsu, China), respectively. All the other chemicals were of analytical grade and double distilled water was used in all studies.

2.2. Phase solubility studies

Phase solubility studies were performed according to the method reported by Higuchi and Connors (1965), at 25 ± 0.5 °C in the dark, using two phosphate sodium buffer solutions of 0.025 mol l^{-1} at pH 4.50 and pH 6.86 and HP-β-CD of varying concentrations (0–0.127 mol l^{-1}). An excess amount of EDA was added to 20 ml of each HP-β-CD concentration solution in 50 ml Erlenmeyer flask and the flask was sealed and shaken at 25 ± 0.5 °C for 12 h. After equilibrium for 2 h, the aliquots (2 ml) were filtered through 0.22 μm filter (Shanghai Bandao Industrial Co. Ltd., China) and appropriately diluted. The concentration of EDA in the filtrate was determined with HPLC. The experiments were carried out in triplicate for each pH buffer. The phase solubility diagram was plotted by using HP-β-CD concentration as X-axis and EDA concentration as *Y*-axis. The stability constants, K_s, were calculated from the straight-line portion of the phase solubility diagram according to the Higuchi–Connors equation (Eq. (1)):

$$K_s = \frac{a}{[S_0 \times (1-a)]} \quad (1)$$

wherein, "*a*" is the slope and "S_0" is the solubility of EDA in the absence of HP-β-CD, which could be obtained from the straight-line of the phase solubility diagram.

2.3. HPLC analysis of EDA

EDA was analyzed with a Shimadzu HPLC System (Shimadzu, Japan) equipped with a Hedera ODS C_{18} column 250 mm × 4.6 mm (5 μm), by using methods similar to those described by Wei and Xiao (2007). The flow rate and the column temperature were 1.0 ml min^{-1} and 35 °C, respectively. Wavelengths and mobile phases were 240 nm and methanol/monopotassium phosphate (0.05 mol l^{-1}, pH 3.5) (50:50, v/v), respectively. The injection volume and the retention time of EDA were 20 μl and 8.5 min, respectively, under our assay conditions. The mean regression equation for EDA was $y = 8946.1x + 5629.2$ ($r = 0.9998$, $n = 10$) in the concentration range of 10.15–101.5 μg ml^{-1}, wherein *y* is the peak area of EDA and *x* is the concentration of EDA. Analytical performance was within acceptable limits with inter-day and intra-day relative standard deviations of less than 5%.

2.4. Preparation of the inclusion complex and physical mixture

An inclusion complex of EDA with HP-β-CD was prepared by freeze-drying method. EDA and HP-β-CD were weighted precisely in the 1:1 molar ratio on the basis of the results obtained from the pilot phase solubility studies. Specifically, the solution of EDA in ethanol was added slowly into the phosphate sodium buffer solution of HP-β-CD. The mixture was magnetically stirred at room temperature for 2 h, and heated at 35 °C for 20 min to thoroughly remove ethanol under reduced pressure. Then the resulting solution was filtered through a 0.22 μm PTFE filter. The filtrate was frozen and then lyophilized for 12 h.

A physical mixture consisting of EDA and HP-β-CD in the same 1:1 molar ratio was prepared. The EDA and the HP-β-CD were added in a mortar and mixed for 10 min to obtain a homogeneous blend. In addition, to eliminate the possible influence of the process on the characteristics of EDA, it was processed in the same way as the complex was prepared. Such EDA was used to prepare the physical mixture with HP-β-CD and used as a control for the physicochemical characterization of the inclusion complex.

2.5. Characterization of EDA/HP-β-CD inclusion complex

2.5.1. Differential thermal analysis

DTA curves of EDA, HP-β-CD, EDA/HP-β-CD inclusion complex and their physical mixture were measured with a DTA-60 differential thermal analyzer (Shimadzu, Japan). Each sample (3–5 mg) was accurately weighed and heated in an aluminum pan at a rate of 10.00 °C min^{-1} between 30 °C and 350 °C temperature range under a constant flow (30 ml min^{-1}) of dry nitrogen. Comparison of the DTA curves afforded some useful information about peak position, peak shifting, and the presence/absence of peaks at certain temperatures.

2.5.2. X-ray powder diffractometry

X-ray powder diffraction patterns were recorded by using a Ricoh Dmax 2500 diffractometer (Ricoh, Japan) with tube anode Cu over the interval 5–45°/2θ. The powder samples were packed in the X-ray holder prior to analysis and the operation data were as follows: generator tension (voltage) 40 kv, generator current 200 mA and scanning speed 2°/min.

2.5.3. NMR spectra

NMR is the most effective method to clarify the formation of an inclusion complex with HP-β-CD. ^{1}H, ^{13}C and 2D (^{13}C–^{1}H, COSY) NMR spectra of EDA and EDA/HP-β-CD complex were recorded with a 400 MHz Bruker AVANCE II spectrometer at 25 °C. Samples were dissolved in D_2O and degassed by bubbling N_2 directly in the NMR tubes. The chemical shifts (δ) were reported as ppm and are referenced to the residual water signal.

2.6. Dissolution rate studies

In vitro dissolution studies of EDA and EDA/HP-β-CD inclusion complex were conducted according to the CP2005 and in a dissolution apparatus (ZRS-8G, Tianjin University, China) using the paddle method at 37 ± 0.5 °C, rotating at 100 ± 2 rpm. Powdered EDA (400 mg) or equivalent inclusion complex with HP-β-CD was added to 100 ml of distilled water. An aliquot (0.5 ml) was withdrawn and replaced with the same volume of fresh medium at predetermined time intervals (1, 2, 5, 10, 20, 30, 40, 50 and 60 min). The solution was immediately filtered (0.22 μm pore size) and

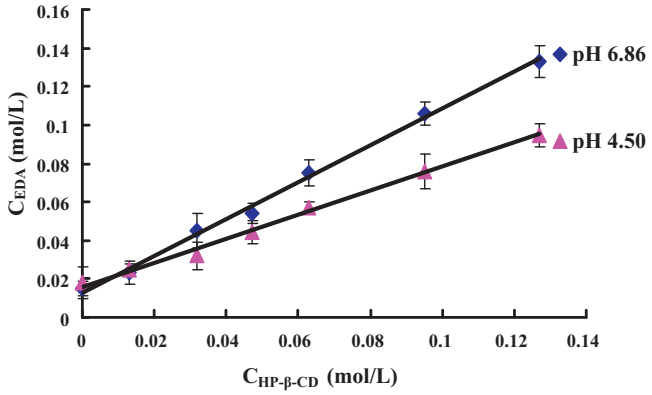

Fig. 2. Phase solubility study of EDA with HP-β-CD in two different pH buffers (25 ± 0.5 °C).

properly diluted. Drug content was analyzed with HPLC at 240 nm. Each experiment was carried out in triplicate.

2.7. Stability studies

Comparative tests involving the stability of free EDA and the inclusion complex were tested in the following conditions: (a) stored in brown glass bottles at a temperature of 40 °C for 10 days; (b) dissolved in 0.9% sodium chloride injection solution for 24 h at room temperature using a water bath. After a fixed period of time, the retention amount of free or complex EDA was measured to evaluate the embedding effect of the inclusion complex.

3. Results and discussion

3.1. Phase-solubility study

The phase solubility technique is very useful for investigating inclusion complexation of poor water-soluble drugs with CDs in water, because it gives not only the solubilizing ability of CDs but also the stability constant of the complexes by analyzing the solubility curve (Higuchi & Kristiansen, 1970).

Fig. 2 shows the phase solubility diagrams of EDA and HP-β-CD in two different pH buffers. The apparent solubility of EDA increased linearly with the concentrations of HP-β-CD. The plot at each pH showed a linear trend, therefore, both of them can be considered as A_L-type diagrams (Higuchi & Connors, 1965), which indicated the formation of 1:1 inclusion complex in the systems.

The stability constants, K_s, of EDA and HP-β-CD in two different pH buffers are summarized in Table 1. The K_s in pH 4.50 buffer was estimated to be 126.6 M^{-1} from the slope and intercept of the linear phase solubility curve, 11-fold smaller than that in pH 6.86 buffer. This result indicated that the ability of inclusion between EDA and HP-β-CD was smaller in acidic solution than that in neutral solution. Meanwhile, the intrinsic solubility of EDA was increased from 0.013 mol ml^{-1} in pH 6.86 buffer to 0.016 mol ml^{-1} in pH 4.50 buffer, which may be attributed to the ionization of EDA ($pK_a = 6.9 \pm 0.1$) at a lower pH.

Table 1
The stability constant (K_s) of EDA at two pH buffer solutions (25 ± 0.5 °C).

pH	Linear equation	R^2	K_s (M^{-1})
4.50	$y = 0.6220\ c + 0.0161$	0.9946	126.6
6.86	$y = 0.9552\ c + 0.0130$	0.9967	1460.1

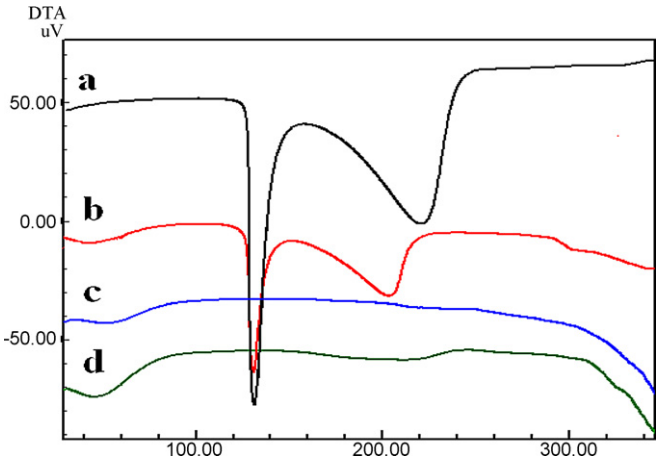

Fig. 3. DTA thermograms of (a) EDA, (b) physical mixture, (c) HP-β-CD and (d) inclusion complex.

3.2. Characterization of the inclusion complex

3.2.1. Differential thermal analysis

It is known that when guest molecules are embedded in the cavity of CD or in the crystal lattice, their melting, sublimating and/or boiling points generally shift to a different temperature or disappear in the case of CD decomposition (Marques, Hadgraft, & Kllaway, 1990). The thermograms of EDA, HP-β-CD, physical mixture and inclusion complex are shown in Fig. 3.

The DTA thermogram of EDA (Fig. 3a) was typical of an anhydrous substance with a sharp melting endotherm peak (T_{onset} = 131.2 °C), followed by an endothermic peak at about 220.0 °C that might be due to the decomposition of the drug. The DTA curve of HP-β-CD alone (Fig. 3c) showed a very broad endothermic effect, between 37.3 °C and 113.4 °C, which attained a maximum at around 51.1 °C corresponding to the dehydration process. Besides, the base shift around 304.2 °C may result from a degradation process of HP-β-CD. The DTA curve of the physical mixture of EDA with HP-β-CD (Fig. 3b) showed the persistence of the endothermic phenomenon due to loss of water, characteristic of HP-β-CD, and the melting peak of the drug. The appearance of EDA melting peak in the physical mixture was indicative of the drug alone. However, in the DTA curve of the EDA/HP-β-CD complex (Fig. 3d), the endothermic peak of free EDA at about 130 °C disappeared completely. This may be attributed to the formation of an inclusion complex between EDA and HP-β-CD, as supported by XRD and especially by NMR (*vide infra*).

3.2.2. X-ray powder diffractometry

Further evidence for the formation of EDA/HP-β-CD complex was obtained from XRD (Fig. 4). XRD is a useful method for the detection of cyclodextrin complexation in powder or microcrystalline states. If an intrinsic inclusion complex is formed, the diffraction pattern of the complex would be clearly distinct from the superposition of each of the components of the system (Nikolic et al., 2004).

The diffraction pattern of EDA (Fig. 4a) displayed intense peaks, indicative of its crystalline character. In contrast, HP-β-CD shown in Fig. 4c was amorphous lacking crystalline peaks. The XRD pattern of the physical mixture mostly showed the characteristic of amorphous cyclodextrins, while some crystallinity peaks of the drug were noticeable (Fig. 4b). However, compared to the diffraction patterns of EDA and HP-β-CD, the lyophilized inclusion complex (Fig. 4d) showed an amorphous structure, probably due to both the structure of HP-β-CD and the lyophilization process. This was an evidence of the absence of EDA crystalline particles, and the

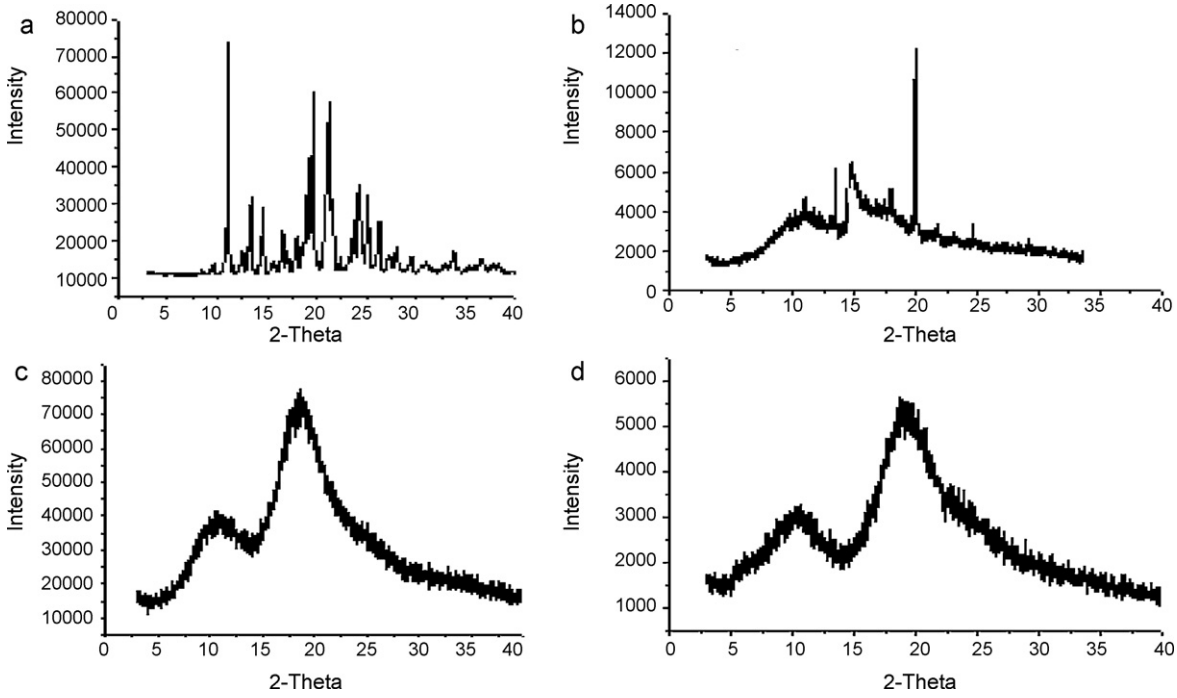

Fig. 4. XRD patterns of (a) EDA, (b) physical mixture, (c) HP-β-CD and (d) inclusion complex.

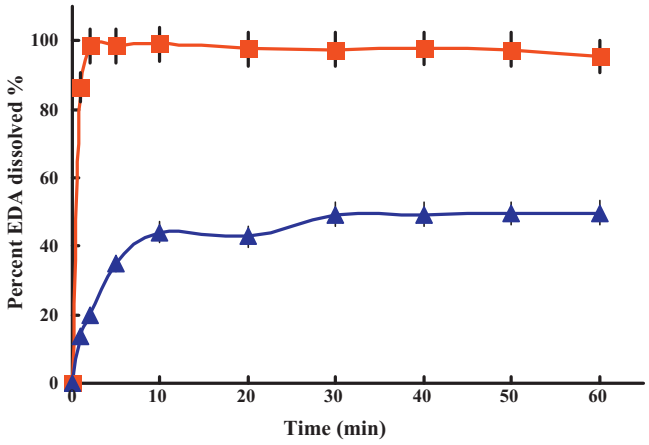

Fig. 5. Dissolution profiles of (▲) EDA and (■) its complex with HP-β-CD.

Table 2
Chemical shift values (ppm) of EDA before and after complexed with HP-β-CD at 25 °C.

Protons	$\delta_{EDA\ free}$	$\delta_{EDA\ complexed}$	$\Delta\delta_{(EDA\ complexed\text{-}EDA\ free)}$
2′ (6′)-H	7.471	7.673	+0.222
3′ (5′)-H	7.328	7.319	−0.009
CH_3–H	2.078	2.126	+0.048
4′-H	7.266	7.243	−0.023
4-H[a]	3.581	3.628	+ 0.047

[a] Overlapped with the signals of HP-β-CD and assigned according to ^{13}C–^{1}H COSY.

XRD patterns were in agreement with the results of DTA, suggesting the formation of an inclusion complex between EDA and HP-β-CD.

3.2.3. NMR spectra

The most direct evidence for the formation of an inclusion complex was from the complexation-induced shifts of EDA in ^{1}H NMR

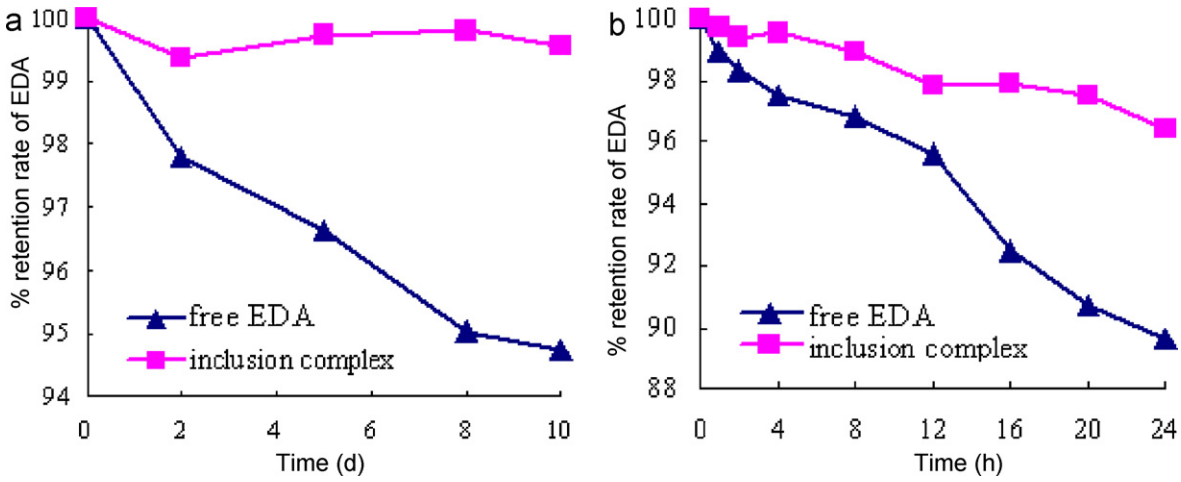

Fig. 6. the stability of EDA and inclusion complex (a) at a temperature of 40 °C; (b) in the 0.9% sodium chloride injection solution at room temperature.

spectra. The ^{1}H chemical shifts of EDA, before and after complexed with HP-β-CD, were shown in Table 2. It can be seen that the signals for 2′ (6′)-H and CH_3–H protons were downfield shifted, whereas upfield shifts were observed for 3′ (5′)-H and 4′-H protons possibly due to the steric effect from HP-β-CD. The signal from 4-H of EDA, which was overlapped with the signals of HP-β-CD at 3.36–3.91 ppm, was also downfield shifted as confirmed by ^{13}C–^{1}H COSY. In addition, the H_3 and H_5 protons of HP-β-CD, located in the interior, were obviously shifted upfield. These chemical shifts clearly demonstrated that EDA formed an intrinsic inclusion complex with HP-β-CD and that it was deeply included in the cavity of HP-β-CD, though the exact orientation of EDA in the cavity needs to be established.

3.2.4. Dissolution rate studies

Fig. 5 shows the dissolution profiles of EDA from EDA and its complex with HP-β-CD. It was evident that the complex exhibited faster dissolution rate than EDA alone. Only about 50% of the powdered drug dissolved in 60 min. In sharp contrast, the complexed EDA completely dissolved within 2 min. The very high increase of EDA dissolution rate in the case of inclusion complex might be due to several reasons: the formation of soluble inclusion complex, amorphization of the drug and consequently solubility increase, better wettability and reduction of particle size (Veiga, Fernandes, & Maincent, 2001).

3.2.5. Stability studies

The stabilities of EDA in samples stored in brown glass bottles at 40 °C and in 0.9% sodium chloride injection solution are shown in Fig. 6(a) and (b), respectively. As shown in Fig. 6(a), effect of heat on the degradation of free or complex EDA was verified at 40 °C. Within 10 days free EDA had a severe degradation of 5.25%, whereas there was no obvious change of the complexed EDA with a decrease of 0.44%. These results strongly suggested that the effect of heat on the inclusion complex was insignificant, thus the inclusion complexation afforded an efficient protection for EDA. So the stability of EDA against heat was greatly enhanced when formed inclusion complex with HP-β-CD.

The stability of EDA in 0.9% sodium chloride injection solution was illustrated in Fig. 6(b). After 12 h, the mean residual level of EDA in the inclusion complex was 97.8% while free EDA had a more severe decrease in 0.9% sodium chloride injection solution. This result indicated that the chemical stability of EDA in aqueous solution was also improved when EDA and HP-β-CD formed an inclusion complex.

4. Conclusion

In conclusion, this study showed that EDA was capable of forming an inclusion complex with HP-β-CD in the stoichiometric ratio of 1:1, resulting in an A_L-type phase-diagram. The structure of the inclusion complex was characterized by DTA, XRD and NMR. Upon complexation with HP-β-CD, the solubility, dissolubility and stability of EDA were greatly enhanced. This result may provide some guidances for the future development of the oral formulation of EDA, which is currently under active investigation in our lab and will be reported in due course.

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