Title : Evaluation of actarit / γ-cyclodextrin complex prepared by different methods

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Abstract

This study used actarit (ACT), an antirheumatic drug, to examine the molecular interaction of ACT and γ-CD in a solid state as a result of cogrinding or freeze-drying and it assessed the dissolution of ACT.

Differential scanning calorimetry (DSC) revealed that coground ACT and γ-CD at molar ratios of 1:2 and 1:3 and freeze-dried ACT and γ-CD at molar ratios of 1:1 and 1:2 lacked an endothermic peak due to melting of ACT crystals. Thus, coground ACT and γ-CD at a molar ratio of 1:2 had molecular interaction, as did freeze-dried ACT and γ-CD at a molar ratio of 1:1. Powder x-ray diffraction (PXRD) revealed that coground and humidified ACT and γ-CD at a molar ratio of 1:2 produced a characteristic diffraction peak at 2θ=15.2° and 16.5° due to the cage structure of γ-CD. In addition, freeze-dried ACT and γ-CD at a molar ratio of 1:1 that had been humidified produced a diffraction peak at 2θ=6.0° and 15.9° characteristic of a hexagonal structure with head-to-head channels due to γ-CD. Assessment of dissolution revealed that ground mixtures (GMs) and freeze-dried mixtures (FDs) had improved dissolution of ACT compared to ACT, ground ACT alone, and physical mixtures (PMs). The mechanism for this is presumably the result of molecular interaction in a solid state or molecular interaction in an aqueous solution. ^{1}H-^{1}H NOESY NMR spectra suggested that in a GM of ACT and γ-CD the benzene ring and methyl group of ACT partially enter the CD cavity. In addition, spectra for freeze-dried ACT and γ-CD suggested that protons of the methylene group of ACT and the benzene ring of ACT partially enter the CD cavity. These findings indicate that ACT and γ-CD inclusion complexes feature different forms of inclusion depending on how they are prepared, e.g. cogrinding or freeze-drying. Findings also indicated that selection of a method of preparation may play a major role in drug development.

Keywords: molecular interaction, ground mixture, freeze dried, γ- cyclodextrin, actarit

Introduction

Various techniques for preparing pharmaceuticals have been researched and developed to improve their bioavailability when they are administered. These varied approaches include control of crystalline structures through the use of polymorphic or amorphous forms [1], use of a solid dispersion [2,3], nanoparticle formation [4], and inclusion complex formation [5]. Inclusion complex formation is a technique that has long been used to improve drug solubility. Molecules such as cyclodextrins (CDs), urea [6], and cholic acid derivatives [7] serve as host molecules, and various compounds are known to be included as guest molecules in those host molecules. CD has been studied and used in various pharmaceuticals to provide cephalosporin and nitroglycerin preparations [8].

CD is, based on the number of glucopyranose units it contains, classified into α-, β-, and γ-CD. CD is widely used as a host molecule because of the inclusion complexes formed by each CD. This trait of CD has been studied and used in an array of pharmaceuticals to improve solubility [9], control release [10], and improve stability [11]. Known methods of preparing inclusion complexes include spray-drying [12], freeze-drying [13], and cogrinding [14]. Cogrinding is a method of preparing samples in a solid state by grinding them together using a vibrating rod mill. This simple and quick method uses samples while they are still in powder form, so stability in solution need not be considered and solubilization need not be done. Ogawa et al. reported that cogrinding resulted in formation of β-CD and fentanyl inclusion complexes [15]. An interesting finding reported by one study is that differences in the structure of inclusion complexes of prostaglandin E1 and α-, β-, or γ-CD result in different properties [16]. The study found that α-CD inclusion complexes enhance solubility, β-CD inclusion complexes enhance stability, and γ-CD inclusion complexes enhance protection from

metabolism of hydroxy groups on the side chain. Thus, the structure of inclusion complexes with CD is known to differ depending on the type of CD and how it is prepared [17].

Actarit (ACT) is a disease-modifying antirheumatic drug (DMARD) that was developed in Japan and that is taken orally. ACT is believed to inhibit chronic inflammation developing in the joints as a result of rheumatoid arthritis [18]. ACT's action is relatively gentle and the drug is widely used in the early to middle stages of rheumatoid arthritis, but it has drawbacks: in clinical practice it causes adverse reactions such as GI disturbances and kidney damage and it lacks solubility in water [19]. The current authors previously prepared ground mixtures using β-CD and ACT and they examined the formation of complexes with β-CD. Results revealed the formation of inclusion complexes with ACT/β-CD channels when the molar ratio of ACT to β-CD was 1:1 [20]. In addition, inclusion of ACT in β-CD resulted in improved solubility of ACT. However, β-CD is barely soluble in water and it readily affects living tissue, so the FAO/WHO Joint Expert Committee on Food Additives (JECFA) has set limits on the maximum amount that can be ingested. Complex formation with other CD derivatives needs to be studied. Thus, the current study examined the interaction between ACT and γ-CD since it is more water-soluble than β-CD and it has little effect on living tissue. By using the ACT / γ-CD inclusion complex effect on the biological tissue low, leading to improved therapeutic effect optimal actarit is expected. Also, by improving the solubility of actarit, and possibility application to injection not only oral formulation, the dosage form of a new selection is advantaged.

The method of preparations chosen were cogrinding and freeze-drying. Various physicochemical properties and dissolution of a ground mixture of ACT and γ-CD and

1 freeze-dried ACT and γ-CD were assessed, and mechanisms for the formation of ACT
2 and γ-CD complexes were examined to look for differences.
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Experimental

Materials and methods

Chemicals

ACT was kindly donated by Nippon Shinyaku Corporation (Kyoto, Japan). γ-CD was donated by Cyclo Chem Co. Ltd (Tokyo, Japan). γ-CD was used after storage at 40°C and RH82% for 3 days. All other chemicals and solvents were of analytical grade.

Preparation of samples

Physical mixtures (PMs) were prepared by mixing γ-CD with ACT at different molar ratios (ACT:γ-CD=1:1, 1:2, and 1:3 for PM1, PM2, and PM3) for 1 minute using a vortex mixer. Ground mixtures (GMs) were prepared by grinding PMs (1.0 g) for 30 min. (GM-30min) using a vibrating rod mill (TI-500ET, CMT Co.) with alumina holder. Freeze-dried mixtures (FDs) were prepared by dissolving γ-CD with ACT in water at different molar ratios (ACT:γ-CD =2:1, 1:1, and 1:2 for FD1, FD2, and FD3). Each FD was briefly frozen at -30°C. A solution was prepared, freeze-dried in a vacuum freeze-dryer (FZ-6, ALS Co., Ltd.) for 24 hours, and then sublimated to remove the solvent.

Preparation of humidified samples

Humidified samples were prepared by storing GMs for 7 days at 40°C and RH82%.

Ground mixture exhibiting an amorphous form instantly changes to a crystalline form when stored at 82% relative humidity, indicating that the ground mixtures, which produce an inclusion complex, would form inclusion complex crystals by absorbing water vapor. In contrast, mixtures containing

no inclusion complex crystallize into their respective component crystals. Thus, these humidified mixtures were used to investigate the interaction between actarit and γ-CD.

Differential scanning calorimetry (DSC)

Thermal behavior of samples was recorded using a differential scanning calorimeter (Thermo plus Evo, Rigaku). All samples were weighed (3-4 mg) and heated at a scanning rate of 5.0°C/min under a nitrogen flow rate of 60 mL/min between 35 and 220°C. Aluminum pans and lids were used for all samples.

Fourier transform infrared (FT-IR) spectroscopy

FT-IR absorption spectra of samples were recorded with a spectrometer (FT/IR-410, JASCO) using the KBr disk method. Scanning was performed over a range of 650–4000 cm^{-1} with a resolution of 4 cm^{-1}.

Powder x-ray diffraction

Powder X-ray diffraction (PXRD) was performed with a powder x-ray diffractometer (MiniFlex II, Rigaku) using Cu $K\alpha_1$ radiation, a voltage of 30 KV, and a current of 15 mA. Sample powders were placed in glass sample holders. Samples were scanned from 3° to 35° (2θ) at a rate 4°/min.

Solid state fluorescence

Solid state fluorescence spectra of samples were measured with an RF-5300PC fluorescence spectrophotometer (Shimadzu Corp.) using a tetrahedral quartz glass holder 5 mm in size, an excitation wavelength of 290 nm, a slit width of 5 nm, an

optical path length, and a fluorescence wavelength range of 290-600 nm.

Dissolution test

Dissolution testing of samples was performed using a dissolution apparatus (NTR-593, Toyama Sangyo) at 37±0.5°C with 900 ml of distilled water that was stirred at 50 rpm using the paddle method. The samples were weighed accurately to be equivalent to 100 mg of ACT. 20 mL of dissolution samples was collected at 1, 3, 5, 10, 15 and 20 min, respectively, through 0.45 μm membrane filters. An equal volume of fresh dissolution media maintained at the same temperature was added after removing the sample to keep the volume of dissolution media constant. 5 mL of filtered sample was diluted to 50 mL with distilled water. ACT concentrations in samples of the diluted solutions were analyzed using UV spectroscopy at 244 nm.

^{1}H-NMR and NOESY

Two-dimensional (2D) nuclear Overhauser effect difference spectroscopy (NOESY) and selective 1D NMR spectroscopy were performed on a Bruker Avance 500 with a TXI probe operating at 699.6 MHz. Spectroscopy conditions were as follows: a pulse width of 90^{o}, a relaxation delay of 6.7 μs, a scan time of 0.500 s, and a temperature of 295 K.

Results and Discussion

Examination of thermal behavior

GMs and FDs were compared using DSC in order to examine the thermal behavior of prepared specimens. Results revealed that ACT crystals and ground ACT alone produced an endothermic peak due to melting of ACT at around 166°C (Fig. 1-a, b). PM1 (ACT:γ-CD=1:1), PM2 (ACT:γ-CD=1:2), and GM1-30 min (ACT:γ-CD=1:1) produced an endothermic peak due to melting of ACT crystals at around 166°C (Fig. 1-e, f, g). However, GM2-30 min and GM3-30 min (ACT:γ-CD=1:2 and 1:3) lacked an endothermic peak due to melting of ACT crystals (Fig.1-h, i).

GM1-30 min (ACT:γ-CD=1:1) presumably had an excess of ACT crystals. According to a study by Kurozumi et al., when CD and a drug had molecular interaction the melting point of the drug disappeared in DSC [21]. The current results similarly revealed that GM2-30 min (ACT:γ-CD=1:2) lacked a peak due to melting of ACT, so some form of molecular interaction between ACT and γ-CD is presumed to have occurred.

FD1 (ACT:γ-CD=2:1) produced an endothermic peak at around 166°C due to melting of ACT crystals, but FD2 (ACT:γ-CD=1:1) and FD3 (ACT:γ-CD=1:2) lacked such a peak (Figs. 1-j, -k, -l). This suggests that FD1 (ACT:γ-CD=2:1) had an excess of ACT crystals.

The aforementioned changes in thermal behavior presumably indicate molecular interaction in a GM or FD of ACT and γ-CD. Coground ACT and γ-CD at a molar ratio of 1:2 and freeze-dried ACT and γ-CD at a molar ratio of 1:1 presumably have molecular interaction.

Examination of molecular interaction in a solid state

DSC revealed molecular interaction of ACT and γ-CD in a mixture of ACT and γ-CD at a molar ratio of 1:2 ground for 30 min and freeze-dried ACT and γ-CD at a molar ratio of 1:1. Thus, FT-IR absorption spectroscopy was performed in order to examine interaction in solid complexes.

FT-IR spectroscopy revealed that ACT crystals and ground ACT alone produced peaks due to the carboxyl group (1695 cm^{-1}), carbonyl group (1637 cm^{-1}), and benzene ring (1602 cm^{-1}) in ACT molecules (Figs. 2-a, -b). PM1 and PM2 (ACT:γ-CD=1:1 and 1:2) similarly had absorption peaks due to the carboxyl group, carbonyl group, and benzene ring in ACT molecules (Figs. 2-e, -f). However, GM2-30min (ACT:γ-CD=1:2) and FD2 (ACT:γ-CD=1:1) produced broadening of the 3 aforementioned peaks due to ACT (Figs. 2-g, -h). This differs from the results for a GM of ACT/β-CD [20]. A GM of ACT and γ-CD and freeze-dried ACT and γ-CD presumably have different molecular interaction than that of a GM of ACT and β-CD.

FT-IR absorption spectroscopy revealed that a GM of ACT and γ-CD and freeze-dried ACT and γ-CD had reduced molecular mobility of ACT, i.e. hydrogen bonds in molecules. This is possibly the result of hydrogen bonds accompanying complex formation by ACT and γ-CD in solid phase.

Examination of molecular states in solids

FT-IR absorption spectroscopy suggested hydrogen bonds accompanying complex formation in a GM of ACT and γ-CD and freeze-dried ACT and γ-CD. Thus, solid-state fluorescence spectroscopy was performed in order to examine molecular states in solid complexes (Fig. 3).

ACT crystals produced a peak at around 358 nm and a small peak (shoulder) at around 368 nm. PM1 and PM2 (ACT:γ-CD=1:1 and 1:2) and FD3 (ACT:γ-CD=1:2)

also produced a peak at around 358 nm. However, GM2-30min (ACT:γ-CD=1:2) produced a peak at a longer wavelength of around 368 nm.

When an electron donor (D) and electron acceptor (A) are both present in the molecules of a DA2 substituted benzene (such as p-dimethylaminobenzonitrile), the substituted benzene is known to have either a planar structure or a twisted structure [22]. If the substituted benzene has a planar structure, it is known to produce a fluorescence emission peak due to locally excited fluorescence at a shorter wavelength. If it has a twisted structure, it is known to produce a fluorescence emission peak known as twisted intramolecular charge transfer (TICT) fluorescence at a longer wavelength. As a result, GMs of ACT and γ-CD often contain ACT with a twisted structure, and this structure presumably differs from that of freeze-dried ACT and γ-CD. Time-resolved fluorometry must be performed to learn more about this.

Examination of the crystalline state

Based on solid-state fluorescence spectroscopy, the molecular structures of a GM of ACT and γ-CD and freeze-dried ACT and γ-CD presumably differ. Thus, PXRD was performed in order to look for potential differences in crystalline states.

ACT crystals and ground ACT alone produced a peak characteristic of ACT at 2θ=13.2° and 18.0° (Figs. 4-a, -b). PM2 (ACT:γ-CD=1:2) produced a diffraction peak due to ACT crystals at 2θ=13.2° and 18.0° and a diffraction peak due to γ-CD at 2θ=12.2° and 16.2° (Figs. 4-a, -c, -e). In contrast, GM2-30min (ACT:γ-CD=1:2) and FD2 (ACT:γ-CD=1:1) lacked a diffraction peak due to ACT crystals and a diffraction peak due to γ-CD; instead, GM2-30min and FD2 produced a halo pattern (Figs. 4-f, -h). Humidified GM2-30min (ACT:γ-CD=1:2) produced a new diffraction peak at 2θ=15.2° and 16.5° while humidified FD2 (ACT:γ-CD=1:1) produced a new diffraction peak at

2θ=6.0° and 15.9° (Figs. 4-g, -i).

These findings indicate that cogrinding and freeze-drying with γ-CD disrupts the regularity of the crystalline lattice in ACT crystals, causing a decline in crystallinity. ACT crystals may have become amorphous or a mechanochemical reaction may have proceeded and those crystals may have become amorphous as the crystalline structure changed to a structure unlike that of ACT. McMullan et al. reported that when CDs form inclusion complexes with low molecular weight compounds, the resulting structure is either a cage structure or a structure with channels [23]. The new peak for humidified GM2-30min (ACT:γ-CD=1:2) coincides with the diffraction peak characteristic of cage structure of γ-CD, as has previously been reported [24], so GM2-30min is presumed to have a cage structure. The new peak for humidified FD2 (ACT:γ-CD=1:1) coincides with the diffraction peak characteristic of a hexagonal structure with head-to-head channels due to γ-CD, as has previously been reported [25], so ACT and γ-CD complexes are presumably formed with a hexagonal structure with head-to-head channels.

Assessment of dissolution

A dissolution test was performed with ACT crystals, ACT ground for 30 min, PM2 (ACT:γ-CD=1:2), GM2-30 min (ACT:γ-CD=1:2), and FD2 (ACT:γ-CD=1:1) to assess the dissolution of ACT. Results indicated that GM2-30 min (ACT:γ-CD=1:2) had the highest dissolution rate, followed by FD2 (ACT:γ-CD=1:1) and then PM2 (ACT:γ-CD=1:2) and ACT crystals. GM2-30 min (ACT:γ-CD=1:2) and FD2 (ACT:γ-CD=1:1) had improved dissolution compared to ACT crystals and ACT ground for 30 min (Fig. 5).

The GMs improved dissolution is not merely the result of grinding ACT but is also

presumably the result of molecular interaction due to cogrinding and freeze-drying of ACT and γ-CD.

Examination of molecular states in solution

Dissolution testing revealed that GMs and FDs had improved dissolution compared to ACT crystals alone, so molecular states in an aqueous solution may have affected that dissolution. Thus, ^{1}H-^{1}H NOESY NMR was performed in order to assess molecular states in an aqueous solution.

GM2-30 min (ACT:γ-CD=1:2) produced cross peaks between the H-2' peak due to the benzene ring of ACT and the H-5 and H-6 peaks of γ-CD and between the H-4' peak due to the methyl group of ACT and the H-5 and H-6 peaks of γ-CD (Figs. 6-a, -b). In addition, FD2 (ACT:γ-CD=1:1) produced cross peaks between the H-2' peak due to the benzene ring of ACT and the H-6 peak of γ-CD and between the H-3' peak due to the methylene group of ACT and the H-6 peak of γ-CD (Figs. 6-c, -d).

These findings suggest that the benzene ring of ACT partially enters the CD cavity in GMs of ACT and γ-CD and that this benzene ring may be located near the wide rim of the CD cavity. In addition, findings suggest that protons of the methyl group of ACT enter the CD cavity and that this methyl group may be located near the wide rim of the CD cavity. In the freeze-dried ACT and γ-CD, protons of the methylene group of ACT and the benzene ring of ACT partially enter the CD cavity, and this benzene ring may be located near the wide rim of the CD cavity. Protons of the methylene group may be located near the narrow rim of the CD cavity [26].

A previous study of a GM of ACT and β-CD indicated that the methylene group of ACT enters the CD cavity and that the benzene ring of ACT partially enters the CD cavity to lie near the wide rim of the CD cavity. This indicates that ACT and γ-CD

complexes feature different forms of inclusion unlike those found in ACT and β-CD complexes.

Conclusion

This study revealed formation of ACT and γ-CD inclusion complexes in a solid state as a result of cogrinding and freeze-drying. These inclusion complexes were noted with coground ACT and γ-CD at a molar ratio of 1:2 and with freeze-dried ACT:γ-CD at a molar ratio of 1:1. Unlike ACT and β-CD inclusion complexes, ACT and γ-CD inclusion complexes were formed at different molar ratios of ACT and γ-CD. Moreover, ACT and γ-CD complexes often have a cage structure in GMs, and most of the ACT molecules in these complexes have a twisted structure. ACT and γ-CD complexes often a hexagonal structure with channels in freeze-dried ACT and γ-CD, and most of the ACT molecules in these complexes have a planar structure. These forms of inclusion were verified by PXRD patterns and solid-state fluorescence spectroscopy, suggesting that the forms of inclusion differ.

Improved dissolution of ACT was noted with GMs and FDs. The mechanisms for this improved dissolution were found to involve multiple factors, such as molecular interaction in a solid state and molecular interaction in an aqueous solution.

The current results indicated that ACT and γ-CD inclusion complexes feature different forms of inclusion depending on how they are prepared, e.g. cogrinding or freeze-drying. Results also indicated that selection of a method of preparation may play a major role in drug development.

In the future, detailed sites of molecular interaction need to be ascertained further and CD derivatives also need to be examined.

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FIGURE LEGEND

Fig. 1. DSC curves of ACT/γ-CD systems.

(a) ACT crystal, (b) ACT ground 30min, (c) γ-CD, (d) γ-CD ground 30min, (e) PM1 (ACT/γ-CD=1/1), (f) PM2 (ACT/γ-CD=1/2), (g) GM1-30min (ACT/γ-CD=1/1), (h) GM2-30min (ACT/γ-CD=1/2), (i) GM3-30min (ACT/γ-CD=1/3). (j) FD1 (ACT/γ-CD=2/1), (k) FD 2(ACT/γ-CD=1/1), (l) FD3 (ACT/γ-CD=1/2).

Fig. 2. FT-IR spectra of ACT/γ-CD systems.

(a) ACT crystal, (b) ACT ground 30 min, (c) γ-CD, (d) γ-CD ground 30 min, (e) PM 1(ACT/γ-CD=1/1), (f) PM 2(ACT/γ-CD=1/2), (g) GM 2-30 min (ACT/γ-CD=1/2), (h) FD2 (ACT/γ-CD=1/1).

Fig. 3. Emission spectra of ACT/γ-CD systems.

ACT crystal, PM (ACT/γ-CD=1/1), PM2 (ACT/γ-CD=1/2), GM2-30min (ACT/γ-CD=1/2), FD1 (ACT/γ-CD=1/1).

Fig. 4. PXRD patterns of ACT-β-CD systems.

(a) ACT crystal, (b) ACT ground 30min, (c) γ-CD, (d) γ-CD ground 30min, (e) PM2 (ACT/γ-CD=1/2), (f) GM2-30min (ACT/γ-CD=1/2), (g) GM2-30min (ACT/γ-CD=1/2) after storage at 40ºC and 82% for 7days, (h) FD 1(ACT/γ-CD=1/1), (i) FD1 (ACT/γ-CD=1/1) after storage at 40ºC and 82% for 7days.

▲:ACT, ●:β-CD, □:cage form, ◇ hexagonal-columnar form

Fig. 5. Dissolution profiles of ACT/γ-CD systems in 900 mL of water (37±0.5ºC) and a stirring 50 rpm.

○:ACT crystal, ●:ACT ground 30min, □:PM 2(ACT/γ-CD=1/2), △:GM2-30min (ACT/γ-CD=1/2), ◇:FD 1(ACT/γ-CD=1/1).

Results were expressed as mean-S.D. (n=3)

Fig. 6-1. ^{1}H-^{1}H NOESY NMR spectrum of FD1 (molar ratio of ACT/γ-CD = 1/1) in D_2O.

(a) X is 7.2-7.5 and the Y axis is 3.5-4.0, (b) X is 2.1-2,2 and the Y axis is 3.5-4.0.

Fig. 6-2. ^{1}H-^{1}H NOESY NMR spectrum of GM2-30min (molar ratio of ACT/γ-CD = 1/2) in D_2O.

(c) X is 7.0-7.4 and the Y axis is 3.4-4.0, (d) X is 1.9-2.1 and the Y axis is 3.4-4.0.

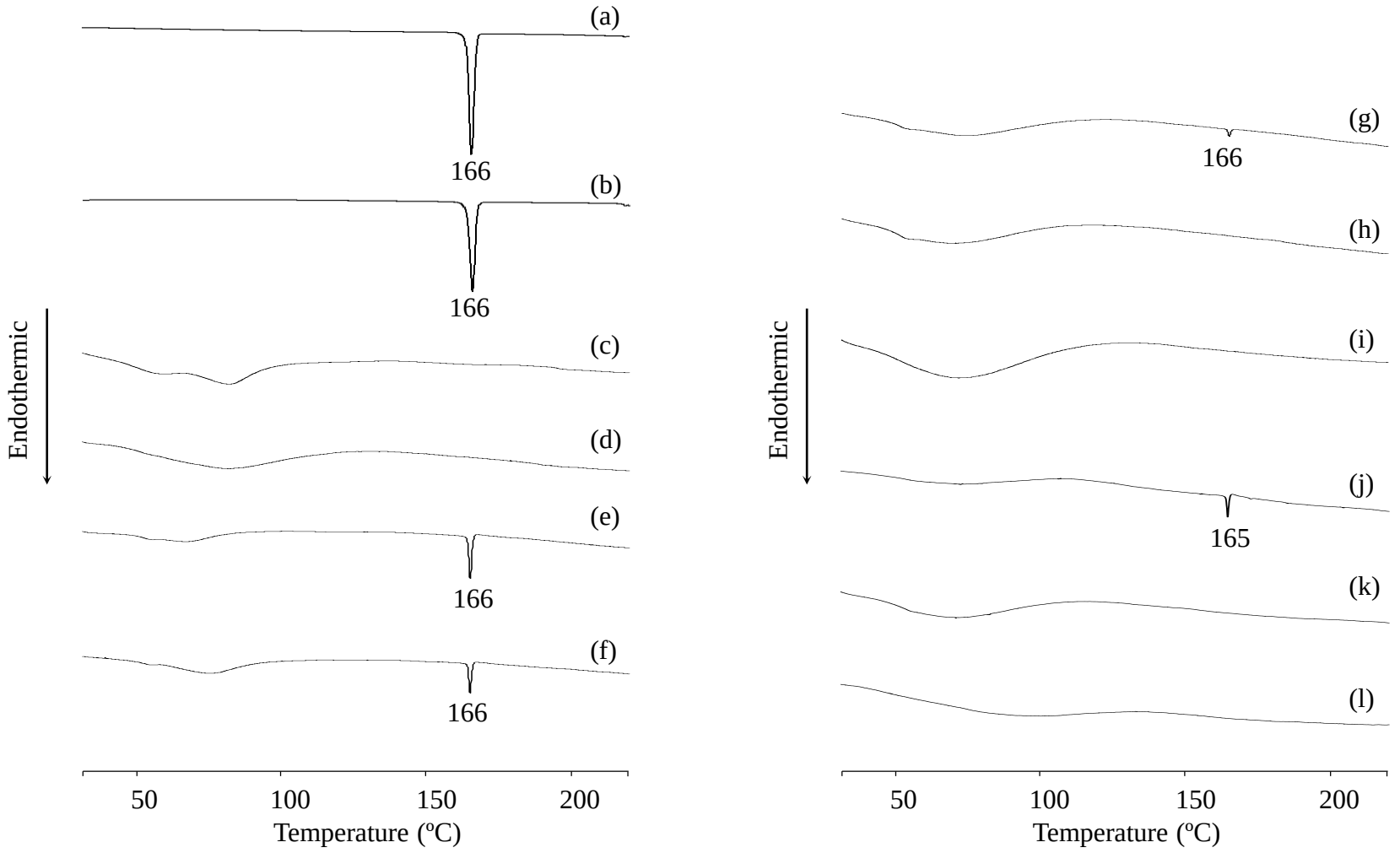

Fig. 1. DSC curves of ACT/γ-CD systems.

(a) ACT crystal, (b) ACT ground 30min, (c) γ-CD, (d) γ-CD ground 30min, (e) PM1 (ACT/γ-CD=1/1), (f) PM2 (ACT/γ-CD=1/2), (g) GM1-30min (ACT/γ-CD=1/1), (h) GM2-30min (ACT/γ-CD=1/2), (i) GM3-30min (ACT/γ-CD=1/3). (j) FD1 (ACT/γ-CD=2/1), (k) FD 2(ACT/γ-CD=1/1), (l) FD3 (ACT/γ-CD=1/2).

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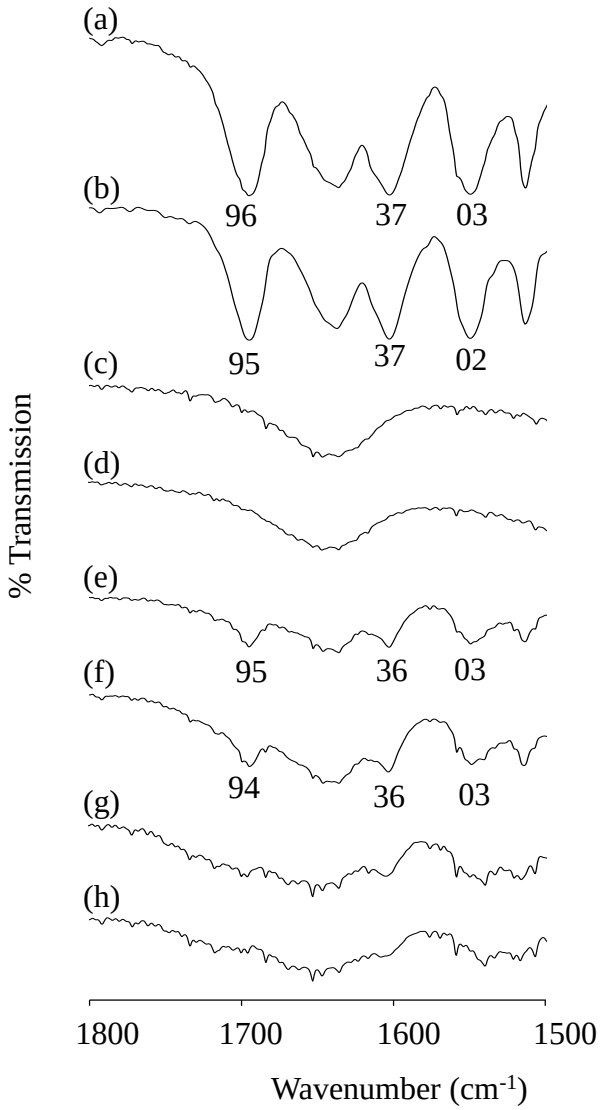

Fig. 2. FT-IR spectra of ACT/γ-CD systems.

(a) ACT crystal, (b) ACT ground 30 min, (c) γ-CD, (d) γ-CD ground 30 min, (e) PM 1(ACT/γ-CD=1/1), (f) PM 2(ACT/γ-CD=1/2), (g) GM 2-30 min (ACT/γ-CD=1/2), (h) FD2 (ACT/γ-CD=1/1).

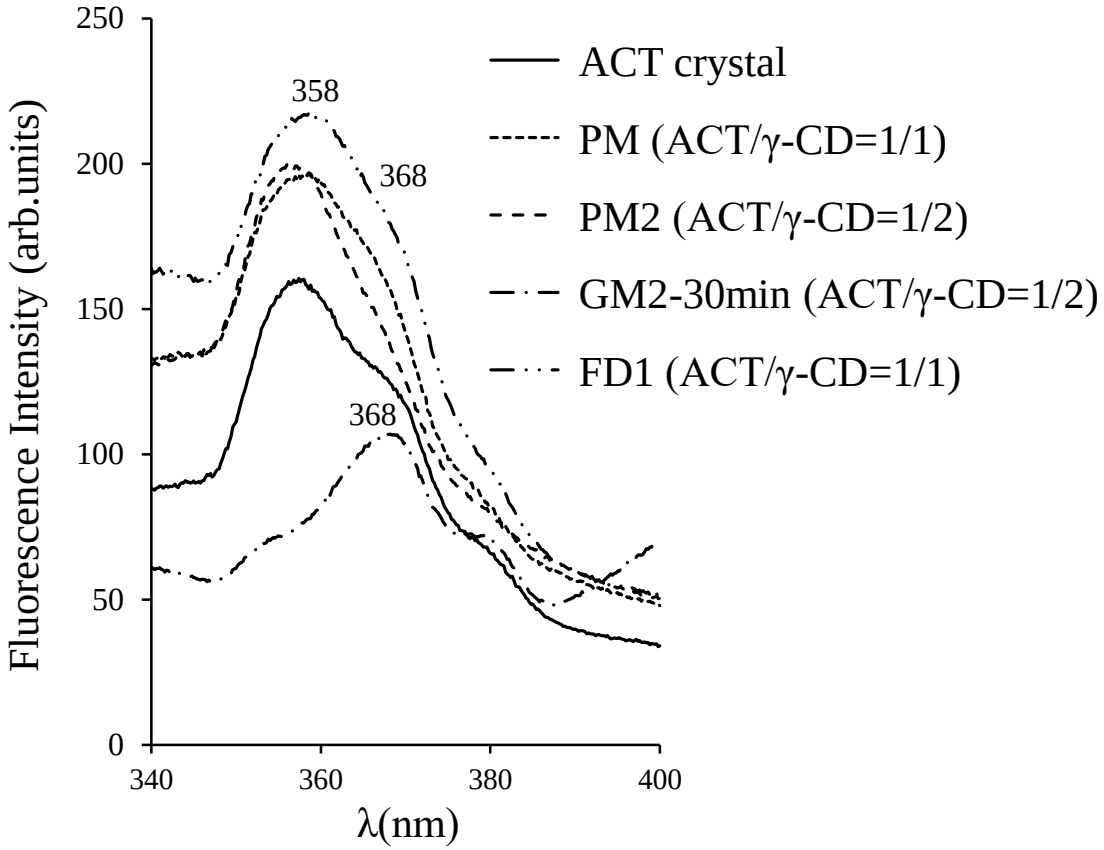

Fig. 3. Emission spectra of ACT/γ-CD systems.

ACT crystal, PM (ACT/γ-CD=1/1), PM2 (ACT/γ-CD=1/2), GM2-30min (ACT/γ-CD=1/2), FD1 (ACT/γ-CD=1/1).

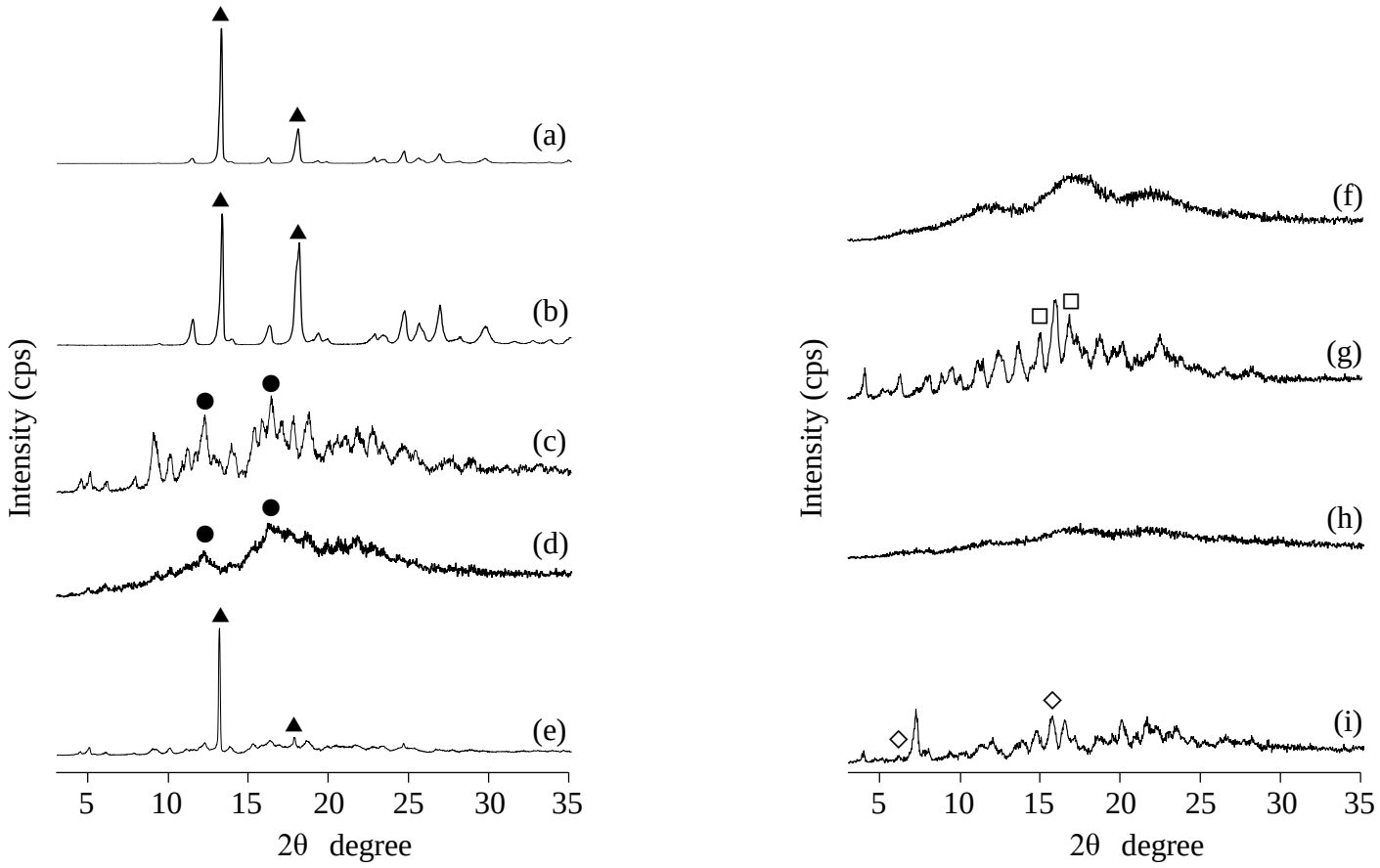

Fig. 4. PXRD patterns of ACT-β-CD systems.

(a) ACT crystal, (b) ACT ground 30min, (c) γ-CD, (d) γ-CD ground 30min, (e) PM2 (ACT/γ-CD=1/2), (f) GM2-30min (ACT/γ-CD=1/2), (g) GM2-30min (ACT/γ-CD=1/2) after storage at 40ºC and 82% for 7days, (h) FD 1(ACT/γ-CD=1/1), (i) FD1 (ACT/γ-CD=1/1) after storage at 40ºC and 82% for 7days.

▲:ACT, ●:β-CD, □:cage form, ◇ hexagonal-columnar form

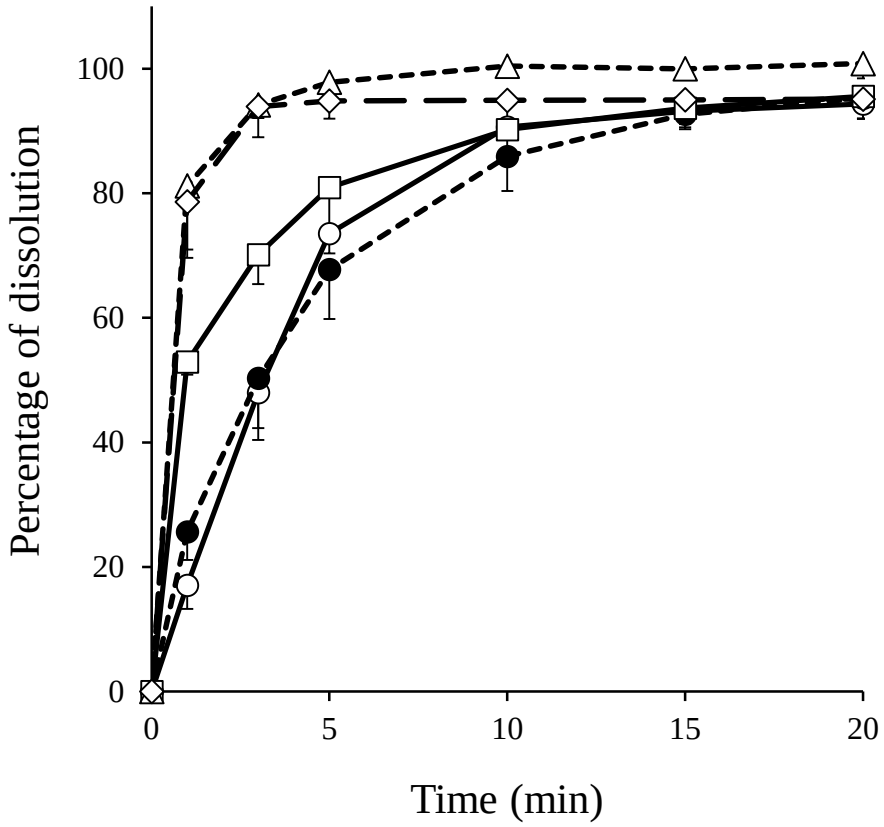

Fig. 5. Dissolution profiles of ACT/γ-CD systems in 900 mL of water (37±0.5ºC) and a stirring 50 rpm.

○:ACT crystal, ●:ACT ground 30min, □:PM 2(ACT/γ-CD=1/2), △:GM2-30min (ACT/γ-CD=1/2), ◇:FD 1(ACT/γ-CD=1/1).

Results were expressed as mean-S.D. (n=3)

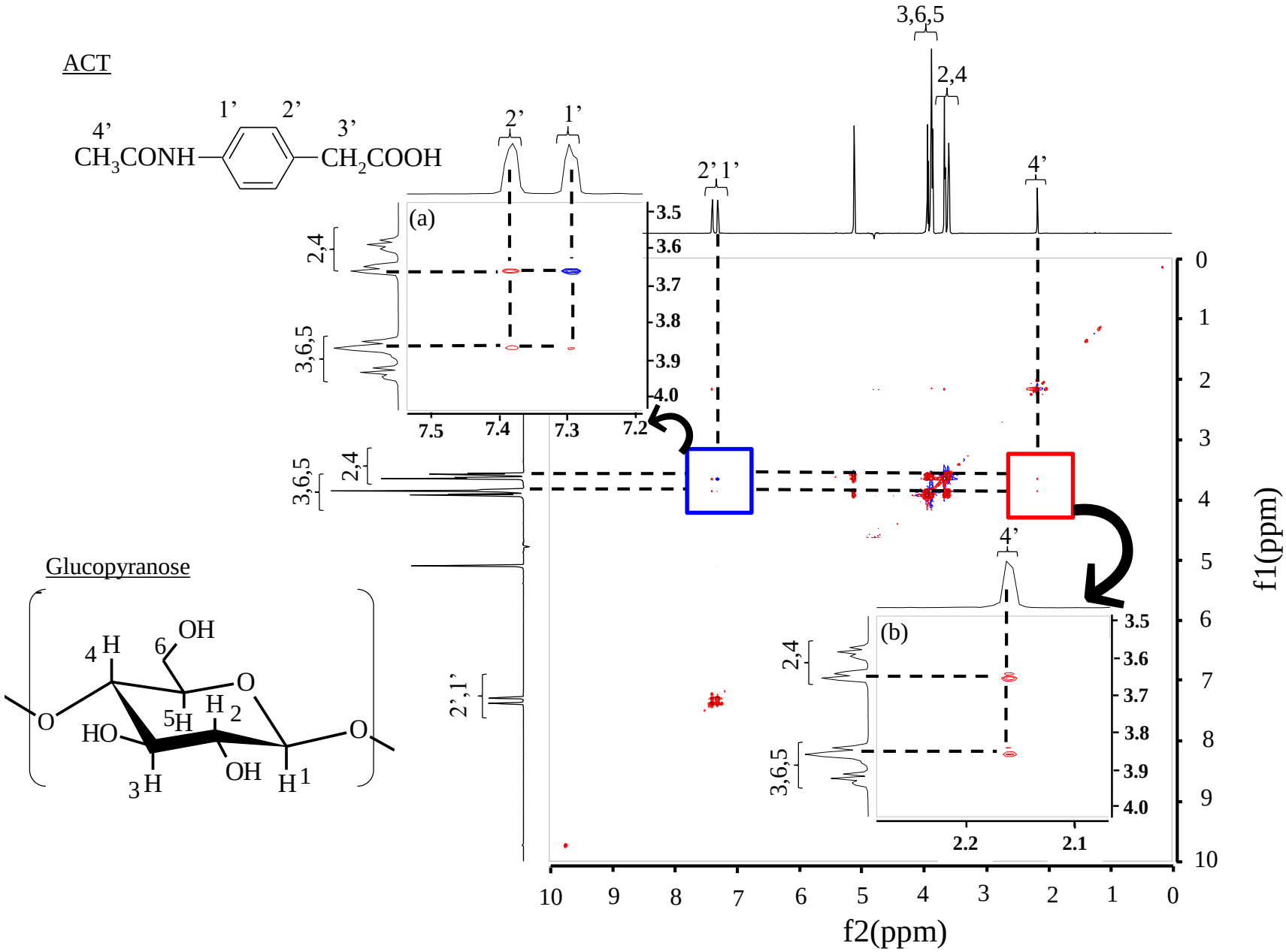

Fig. 6-1. ^{1}H-^{1}H NOESY NMR spectrum of FD1 (molar ratio of ACT/γ-CD = 1/1) in D_2O.

(a) X is 7.2-7.5 and the Y axis is 3.5-4.0, (b) X is 2.1-2,2 and the Y axis is 3.5-4.0.

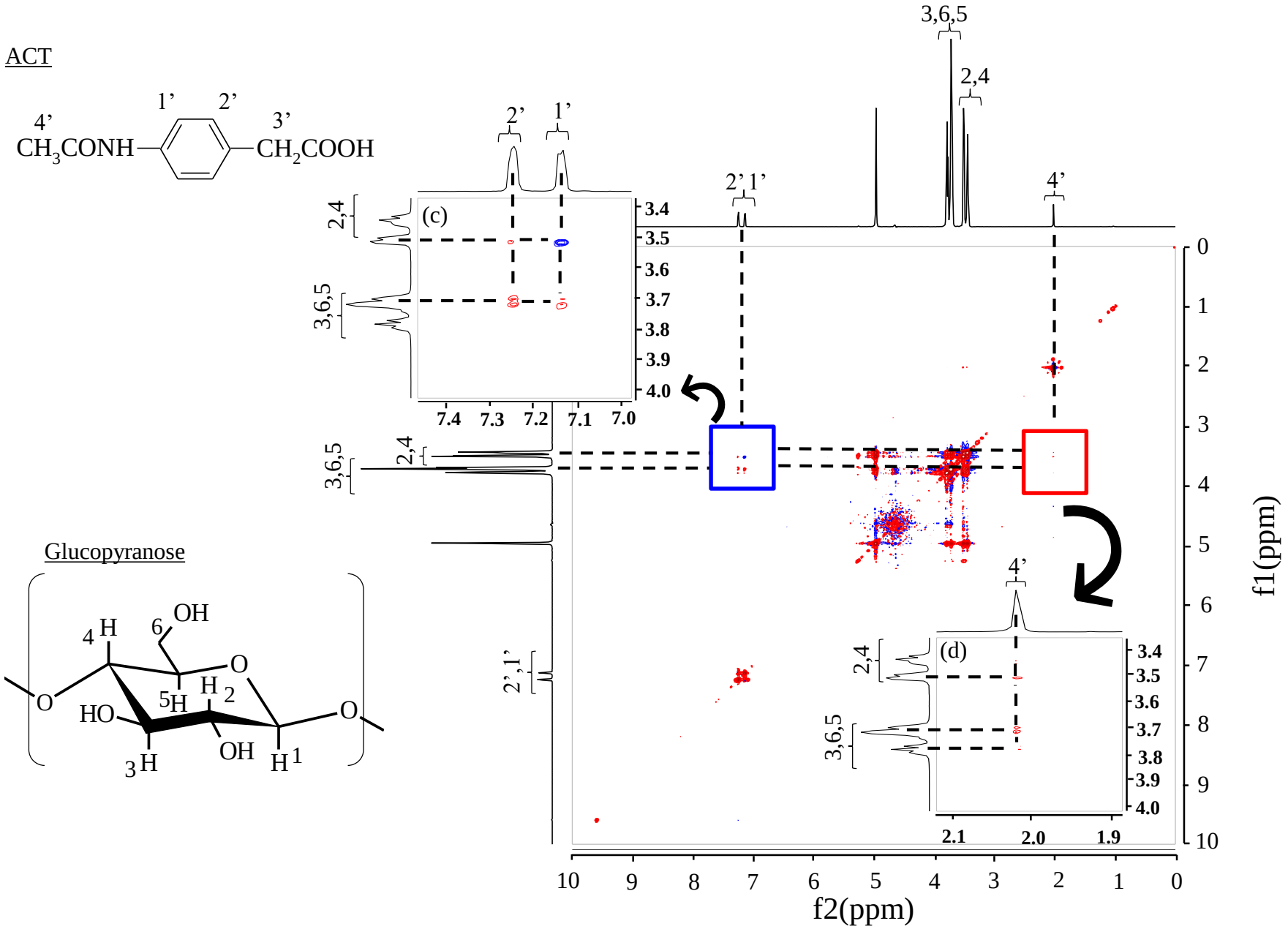

Fig. 6-2. ^{1}H-^{1}H NOESY NMR spectrum of GM2-30min (molar ratio of ACT/γ-CD = 1/2) in D_2O.

(c) X is 7.0-7.4 and the Y axis is 3.4-4.0, (d) X is 1.9-2.1 and the Y axis is 3.4-4.0.