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Alternative oral exemestane formulation: Improved dissolution and permeation

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ABSTRACT

Exemestane (EXE) is an irreversible aromatase inactivator used for the treatment of advanced postmenopausal breast cancer. EXE is orally active but its bioavailability is about 5% due to its low solubility in water and the extensive first pass effect. It is known that cyclodextrin (CD) complexation enhances solubility and oral bioavailability of poorly soluble drugs. Thus, it was aimed to design and develop cyclodextrin complexes in powder and tablet forms containing EXE to improve aqueous solubility and *in vitro* permeability. In this study, inclusion complexes of EXE were prepared with three different CD derivatives (methyl-β-cyclodextrin, hydroxypropyl-β-cyclodextrin and hydroxypropyl-γ-cyclodextrin) and by two different preparation methods (kneading and colyophilization) and the complexes were characterized with ^{1}H NMR, FT-IR, SEM, X-ray and DSC analyses. Both inclusion complexes and tablet formulations prepared using EXE:CD inclusion complexes showed significant improvement in the dissolution profile of this oral antiestrogen drug. Furthermore, Caco-2 cell permeation studies revealed that apparent permeability constant for EXE was increased by 3-fold via cyclodextrin complexation. In conclusion, complexation of EXE with cyclodextrin derivatives, randomly methylated-β-cyclodextrin in particular, results in a more efficient tablet formulation with improved dissolution and better permeation suggesting an enhancement in oral bioavailability of the drug.

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1. Introduction

One-third of human breast tumors are reported to be hormone-dependent and estrogens are the most important hormones involved in the growth of these tumors (Segaloff, 1978; Kirschner, 1979; Theobald, 2000). Estrogen suppression is an important approach to the management of hormone-responsive cancer. Therefore, aromatase inhibition is a well-established therapeutic option in postmenopausal, hormone-dependent breast cancer (Santen et al., 1978; Miller and Dixon, 2002). Exemestane (EXE) is an irreversible steroidal aromatase inactivator; it has been recently approved by the Food and Drug Administration (FDA) for the treatment of breast cancer and marketed as Aromasin® tablet formulation (Johannessen et al., 1997; Valle et al., 2005). This drug is orally active and a potent inhibitor of peripheral aromatase activity (Lonning, 1998).

EXE is a neutral compound with steroidal structure characterized by high lipophilicity. Chemical structure of EXE is given in Fig. 1. It is freely soluble in N,N-dimethylformamide, soluble in methanol, and practically insoluble in water (80 μg/mL). Following oral administration of radiolabeled EXE, 42% of radioactivity was reported to be absorbed from the gastrointestinal tract due to low solubility and first pass effect. Preclinical data obtained in rats and dogs, in which EXE was given intravenously, indicated that the absolute bioavailability was about 5% (FDA NDA, 20753). The Biopharmaceutics Classification System (BCS) is a scientific framework for classifying drug substances based on their aqueous solubility and intestinal permeability. According to the BCS, aqueous solubility and permeability are the most important parameters affecting drug bioavailability. EXE is a BCS Class IV Drug, with characteristic poor aqueous solubility and low permeability (Löbenberg and Amidon, 2000; Yavuz et al., 2007a).

Cyclodextrins (CDs) are cyclic oligosaccharides consisting of D-(+) glucopyranose units which are attached by α-(1, 4) glucosidic bonds. They are capable of forming inclusion complexes with a variety of guest molecules owing to their special structure, with a hydrophilic external surface and a hydrophobic cavity lined with protons. CDs have the ability to interact with poorly water-soluble drugs and drug candidates resulting in an increase in the drug's apparent water solubility and dissolution rates. It is also reported in the literature that CD complexation enhances oral bioavailability of poorly soluble drugs (Albers and Müller, 1995; Loftsson and Brewster, 1996; Thompson, 1997; Challa et al., 2005). The increase in solubility also affects dissolution rate and thus improves oral bioavailability. Through cyclodextrin complexation, it is possible to move Class II drugs, and sometimes even Class IV drugs, into Class I (Loftsson, 2002). Cyclodextrins can be considered practically non-toxic upon oral administration due to lack of CD absorption through

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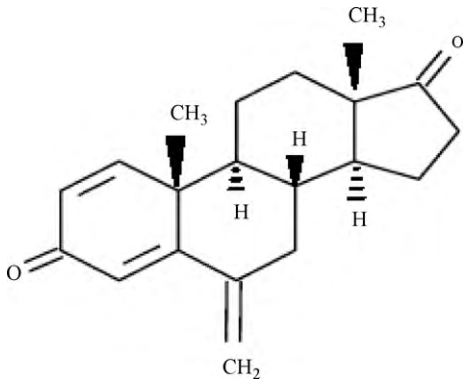

Fig. 1. Chemical structure of exemestane.

gastrointestinal tract as they are known to be absorbed extensively in the colon (Challa et al., 2005; Brewster and Loftsson, 2007).

In this study, the objective was to improve water solubility and intestinal permeability of EXE for a potential enhancement of the oral bioavailability of EXE formulation. To achieve this goal EXE's inclusion complexes were prepared and characterized using different CD derivatives and different preparation techniques and consequently a new tablet formulation was developed using the inclusion complex with optimal dissolution and permeation properties in order to improve EXE's low aqueous solubility and oral bioavailability. All inclusion complexes were comparatively evaluated in terms of complexation abilities, increase in solubility, dissolution enhancement, and effect on *in vitro* permeation through Caco-2 cells. A tablet formulation was developed and compared to commercial Aromasin® tablet formulation for dissolution profiles.

2. Materials and methods

2.1. Materials

EXE and Aromasin® tablets were kind gifts from Pfizer (USA and Turkey). Methyl-β-cyclodextrin (M-β-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD) and hydroxypropyl-γ-cyclodextrin (HP-γ-CD) were supplied as Cavasol® W7M, W7HP and W8HP, respectively, as kind gifts of Wacker Chemie, Germany. Sodium lauryl sulphate (SLS) was purchased from Merck (Germany). High performance liquid chromatography (HPLC) grade acetonitrile and methanol were purchased from Sigma–Aldrich (Germany). Ultrapure water was obtained from Millipore Simplicity 185 (France). Sodium starch glycolate, magnesium stearate and Avicel® pH 102 were obtained from Eczacıbaşı (Turkey). Human colon adenocarcinoma cell line (Caco-2) was used for permeation studies. Caco-2 cell line was obtained from Foot-and-Mouth Disease Institute of Ministry of Agriculture & Rural Affairs of Turkey. Fetal bovine serum (FBS), trypsin–EDTA, Hanks Balanced Salt Solution (HBSS), L-glutamine, RPMI-1640 (without L-glutamine) and penicillin–streptomycin were purchased from Biochrom (Germany). All other reagents were of HPLC grade and were used without purification.

2.2. Methods

2.2.1. Phase solubility studies

0, 5, 10, 15, 20, 25, 30% (w/v) solutions of M-β-CD, HP-β-CD and HP-γ-CD in distilled water were added to separate vials. Excess EXE was added to aqueous solutions containing various concentrations of CDs, which were then stirred at room temperature until equilibrium was reached (7 days). 1 mL CD/drug suspension from each vial was filtered through 0.45 μm polycarbonate membrane filter. The experiments were performed with three replications. The filtrates were quantitatively analyzed by HPLC (Agilent 1100, Germany) with a method developed for EXE, which was validated previously (Yavuz et al., 2007b). Phase solubility diagrams were prepared by plotting cyclodextrin concentration versus solubilized drug concentration. The apparent stability constants ($K_{1:1}$) of the complexes were calculated by the Connors Method (Higuchi and Connors, 1965) using the slope of the 1:1 complex type phase solubility curve as shown in Eq. (1).

$$K_{1:1} = \frac{\text{Slope}}{(1 - \text{Slope}) \times \text{Sw}} \quad (1)$$

Sw is the intrinsic solubility.

2.2.2. Preparation of inclusion complexes

The inclusion complexes of EXE with either M-β-CD, HP-β-CD or HP-γ-CD were prepared by two alternative techniques; kneading and colyophilization methods (Liu and Suyan, 2006) and the molar ratio of EXE:CD was maintained as 1:1 in both methods. Physical mixtures were also prepared by mixing appropriate amounts of EXE and cyclodextrin in a mortar.

2.2.2.1. Kneading method. Corresponding amounts of EXE and CDs with 1:1 molar ratio were accurately weighed. A homogenous paste was prepared by mixing CD, EXE and a small amount of water in a mortar. The paste was further kneaded for 30 min. The obtained masses were dried at 40 °C in an oven for 4 h.

2.2.2.2. Colyophilization method. Corresponding amounts of EXE and CDs with 1:1 molar ratio were accurately weighed. Saturated CD solutions were prepared with CDs and water. Then, EXE solution in methanol was added slowly and a suspension was formed. The suspension was stirred at room temperature for 24 h. Then methanol was evaporated for 2 h and obtained mass was frozen and then lyophilized to obtain EXE:CD complex as dry powder.

2.2.3. Characterization of inclusion complexes

2.2.3.1. Fourier transform infrared (FT-IR) spectroscopy. Fourier transform infrared (FT-IR) spectra of EXE, M-β-CD, HP-β-CD, HP-γ-CD, EXE:CDs inclusion complexes and EXE:CDs physical mixtures were taken with a Perkin-Elmer BX FT-IR spectrophotometer (USA) using previously prepared discs of each sample and potassium bromide containing 0.01 g of sample in 0.1 g of potassium bromide between 800 and 4000 cm^{-1}.

2.2.3.2. X-ray diffractometry. X-ray analyses were performed on EXE, M-β-CD, HP-β-CD, HP-γ-CD, EXE:CDs inclusion complexes and EXE:CDs physical mixtures (Rigaku X-ray Diffractometer, USA) using Ni-filtered Cu Kα (1.542 Å) radiation (40 kV, 40 mA). Powder samples were mounted on a sample holder and scanned from 2° to 50° in 2θ at a speed of 0.02°/min.

2.2.3.3. Differential scanning calorimetry (DSC). Differential scanning calorimetry (DSC) analyses were performed on EXE, M-β-CD, HP-β-CD, HP-γ-CD, EXE:CD inclusion complexes and EXE:CDs physical mixtures with a DuPont DSC 910 Instrument (DuPont, USA) differential scanning calorimeter. Samples weighing approximately 3 mg were heated in hermetically sealed aluminum pans at a rate of 10 °C/min, between 25 °C and 300 °C in a dynamic nitrogen atmosphere.

2.2.3.4. ^1H NMR spectroscopy. Proton nuclear magnetic resonance (^1H NMR) spectra of EXE, M-β-CD, HP-β-CD, HP-γ-CD, EXE:CD inclusion complexes and EXE:CD physical mixtures were taken by a Varian digital FT-NMR spectrophotometer (USA) at 400 MHz. Chemical shifts were given to external tetramethylsilane at 0 ppm

with calibration using solvent signals (methanol at 4.9 ppm and water at 4.75 ppm).

2.2.3.5. Scanning electron microscopy (SEM). A SEM (Jeol-SEM ASID-10, 80 KV, Japan) device was used to evaluate surface characteristics of the inclusion complexes. EXE, M-β-CD, HP-β-CD, HP-γ-CD, EXE:CD inclusion complexes and EXE:CD physical mixture samples were mounted on the metal stubs with conductive silver paint and then coated with a 150 Å thick layer of gold in a Bio-Rad sputter apparatus. SEM images of the samples were obtained at different magnifications.

2.2.4. Dissolution study of inclusion complexes

The dissolution rates of EXE and EXE complexes with M-β-CD, HP-β-CD, HP-γ-CD prepared by both kneading and colyophilization methods were measured. Dissolution experiments were carried out with six replications on powder samples, using a Sotax (USA) dissolution test apparatus in the following media: 0.5% SLS solution and distilled water both at 37 °C by the paddle method at a rotation speed of 100 rpm. Each sample equivalent to 25 mg of EXE was added to 900 mL dissolution medium where the sink conditions were ensured for FDA recommended medium. At appropriate time intervals, 5 mL of the mixture was withdrawn and filtered through a 0.45 μm polycarbonate filter. The initial volume of dissolution medium was maintained by adding 5 mL of fresh medium. Filtrate was analyzed by HPLC.

2.2.5. Preparation of tablet formulation

A tablet formulation was developed with EXE:M-β-CD kneaded complex to compare with Aromasin® tablet in the terms of their dissolution rates. Since *in vitro* characterizations of the complexes as well as phase solubility and dissolution studies showed M-β-CD and kneading method giving optimum results, EXE:M-β-CD kneaded complex was used in the tablet formulation. No solubility enhancer was added to the formulation. The tablets were prepared by direct compression method. The tablets were compressed using 12 mm flat faced punch on a single stroke punching machine. The formulation of the tablet is given below:

EXE:M-β-CD (1:1) kneaded complex	161.7 mg (25 mg EXE)
Avicel pH 102	200 mg
Sodium starch glycolate	32 mg
Mg stearate	8 mg

2.2.6. Dissolution study of tablets

Dissolution studies for EXE:M-β-CD tablets and Aromasin® tablets were carried out with six replications, using a Sotax (USA) dissolution test apparatus in both 0.5% SLS solution and distilled water at 37 °C by the basket method at a rotation speed of 100 rpm. Tablets were added to the 900 mL dissolution medium. At appropriate time intervals, 5 mL of the mixture was withdrawn and filtered through a 0.45 μm polycarbonate filter. The initial volume of dissolution medium was maintained by adding 5 mL of fresh medium. Filtrate was analyzed by HPLC. Cumulative percentages of the drug dissolved from the tablets were calculated. The difference factors (f_1) and the similarity factors (f_2) were calculated to compare dissolution profiles. The difference factor, f_1, was determined with Eq. (2) and the similarity factor, f_2 is defined in Eq. (3). f_1 Value between 0 and 15 and f_2 value between 50 and 100 suggests the two dissolution profiles are similar (Mauger et al., 1986; Moore and Flanner, 1996).

$$f_1 = \left\{ \frac{\sum |R_t - T_t|}{\sum R_t} \right\} \times 100 \quad (2)$$

$$f_2 = 50 \log \left(1 + \frac{1}{n} \sum (R_t - T_t)^2 \right)^{-0.5} \times 100 \quad (3)$$

where n is the number of dissolution sample times, R_t and T_t are the percent drug dissolved at each time point for the test and reference products, respectively.

2.2.7. Transport/permeability studies with Caco-2 cells

Caco-2 cell culture studies were performed on EXE, EXE:CD inclusion complexes which were prepared by kneading method with both M-β-CD, HP-β-CD and HP-γ-CD. Caco-2 cells medium was prepared by adding fetal bovine serum, penicillin–streptomycin and L-glutamine to RPMI-1640 to have the final concentration of 10% (v/v), 50 Unit/mL and 50 μg/mL, respectively.

Cells were seeded at 60,000 cells per well apically in the 12-well inserts and then incubated at 37 °C in 5% CO_2. The medium of the flask was changed with fresh medium after every 2 days and trypsinized when near to confluency. Experiments were performed between 18 and 20 days after seeding when the cell monolayer had reached confluence. Cell monolayer integrity was tested by measuring transepithelial resistance with Millicel® ERS. When the resistance reached in the range of 400–600 Ω cm^2, cell monolayer was used for transport studies.

Culture medium was replaced from each well by 0.5 mL and 1 mL Hank's Balanced Salt Solutions (HBSS) in the apical and basolateral side of the well and the cell monolayers were subsequently equilibrated for 30 min at 37 °C. The highest strength of approved EXE dose is 25 mg. Considering the usual amount of water (250 mL) with which a tablet dose is taken, EXE or its CD complexes were applied to the Caco-2 cells as 100 μg/mL. EXE and EXE:CD complexes solutions in HBSS containing 1% DMSO (0.5 mL) were added to the apical side of the monolayer and 1 mL HBSS was used in the basolateral side. The wells were then placed on a shaker at 30 rpm and 37 °C for 2 h after which samples from the basolateral side was analyzed by HPLC.

Apparent permeability co-efficient (cm/s) was calculated by Eq. (4).

$$P_{app} = \frac{dC}{dt} \times \frac{1}{AC_0} \quad (4)$$

where dC/dt is the rate of drug permeation (μg/s); A is the surface area of the insert (cell monolayer) (cm^2); C_0 is the initial concentration of drug in the apical side (μg/mL).

Statistical analysis of data was performed by Kruskal–Wallis one-way Variance Analysis. Post-hoc comparison of means was performed by Kruskal–Wallis test with post-hoc procedures. Statistical significance was considered at $P < 0.05$.

3. Results and discussion

In this study, inclusion complexes of EXE with M-β-CD, HP-β-CD and HP-γ-CD were prepared by two different methods to compare their complexation abilities, solubility, dissolution and permeability enhancer effects. A tablet formulation was developed using the optimum inclusion complex to improve EXE's low aqueous solubility and oral bioavailability.

The determination of the phase solubility diagram is a widely accepted method for evaluation of the effect of CD complexation on the drug solubility. The 1:1 drug/cyclodextrin complex is the most common type of association where a single drug molecule is included in the cavity of one cyclodextrin molecule, with a stability constant $K_{1:1}$ for the equilibrium between the free and associated species (Davis and Brewster, 2004). Corresponding solubility diagrams of EXE with M-β-CD, HP-β-CD and HP-γ-CD are presented, respectively, in Fig. 2. Solubility diagrams for both EXE-M-β-CD, EXE-HP-β-CD and EXE-M-γ-CD are of the AL type which means linear increases of drug solubility as a function of CD concentration similar to that reported by Higuchi and Connors (1965). From

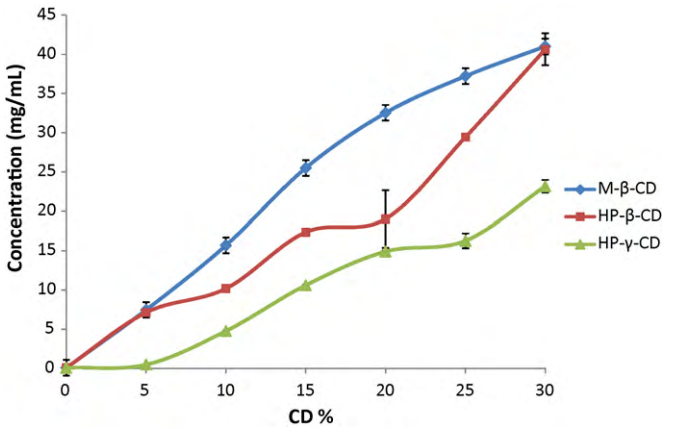

Fig. 2. Phase solubility diagram of EXE with CDs in distilled water ($n = 3$, ±SD).

the phase solubility diagrams, it can be said that the solubility of EXE increases linearly with increasing concentration of various CDs. The apparent 1:1 stability constant ($K_{1:1}$), calculated from the initial straight-line part of the solubility curves was approximately 3700 M^{-1} for all CD derivatives which indicates a similar interaction between the drug and cyclodextrin derivatives in the conditions used in the study. On the other hand, solubility enhancement of different CD derivatives for EXE was calculated to be 513-, 508- and 290-fold indicating efficient solubility improvement for M-β-CD and HP-β-CD complexes in particular.

Inclusion complexes of EXE with M-β-CD, HP-β-CD and HP-γ-CD were characterized by FT-IR spectroscopy, X-ray diffractometry, DSC, ^{1}H NMR spectrometry and SEM imaging. FT-IR spectra of EXE, M-β-CD, HP-β-CD, HP-γ-CD, EXE:CDs inclusion complexes and EXE:CDs physical mixtures with these CD derivatives are presented in Fig. 3. FT-IR spectra of the complexes indicated the disappearance of typical bands of EXE such as C=C stretch absorption (transmittance) peak at 1590–1640 cm^{-1}, C=O stretch peak at 1650–1740 cm^{-1}. C–H stretching regions are present in both EXE and CDs structures, thus disappearance of C–H stretch peak was not expected upon complexation. The O–H stretching bands are typical bands for cyclodextrins, but any shifts of these bands might indicate the formation of hydrogen bonds between EXE and cyclodextrin. These results indicate that a chemical interaction between EXE and corresponding CD due to inclusion in CD cavity especially more pronounced with kneading method.

Further evidence of complex formation was obtained by X-ray powder diffraction. X-ray diffractograms for both M-β-CD, HP-

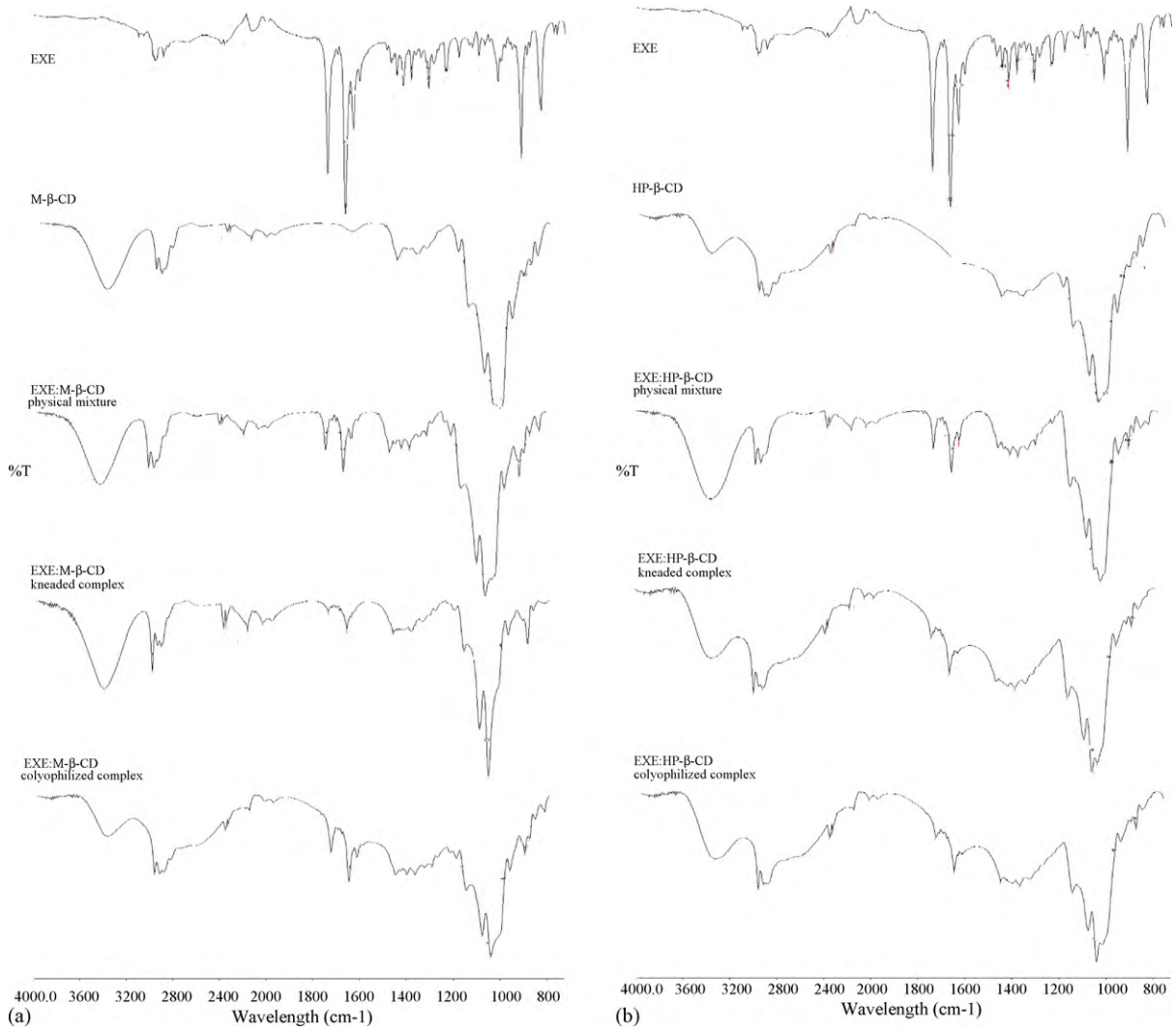

Fig. 3. FT-IR spectrums of EXE, CDs, inclusion complexes and physical mixtures (a: EXE, M-β-CD, EXE:M-β-CD physical mixture, EXE:M-β-CD kneaded complex and EXE:M-β-CD colyophilized complex; b: EXE, HP-β-CD, EXE:HP-β-CD physical mixture, EXE:HP-β-CD kneaded complex and EXE:HP-β-CD colyophilized complex; c: EXE, HP-γ-CD, EXE:HP-γ-CD physical mixture, EXE:HP-γ-CD kneaded complex and EXE:HP-γ-CD colyophilized complex).

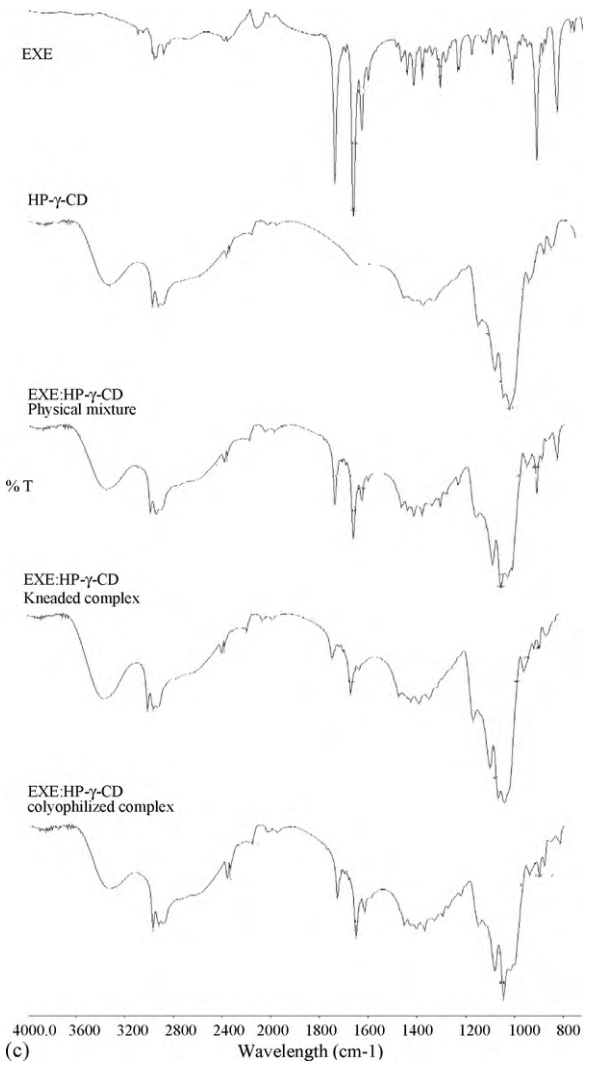

Fig. 3. (*Continued*).

β-CD and HP-γ-CD, EXE:CDs inclusion complexes and EXE:CDs physical mixtures with these derivatives are presented in Fig. 4. Disappearance of free EXE crystal peaks indicates that EXE's crystal properties are masked due to complexation. Inclusion complexes are dynamic systems which molecules change positions continuously according to their apparent stability constant ($K_{1:1}$). Thus there can be seen free crystalline EXE in the diffractograms which is related to the complexation efficiency.

The DSC result of EXE demonstrates an endothermic peak for EXE at 194 °C, which corresponded to the melting point. Disappearance of EXE's characteristic melting endotherm that appears at 194 °C indicates the absence of free crystalline EXE. DSC thermograms of EXE, CDs and EXE:CD complexes are seen in Fig. 5. As can be seen in Fig. 5a melting endotherms of EXE disappear totally in all the M-β-CD formulations. Fig. 5c also shows that the melting endotherm of EXE has been significantly reduced in the EXE:HP-γ-CD inclusion complexes. This may suggest that EXE exists in free form in trace amounts in the complex. These results were consistent with the results obtained from X-ray, FT-IR, ^{1}H NMR, SEM and the phase solubility study.

^{1}H NMR is one of the most selective tools for the characterization of inclusion complexes and for the demonstration of total or partial inclusion in the cyclodextrin cavity. Chemical shift variations of specific host or guest nucleus could provide evidence for the formation of inclusion complexes in solution, since significant

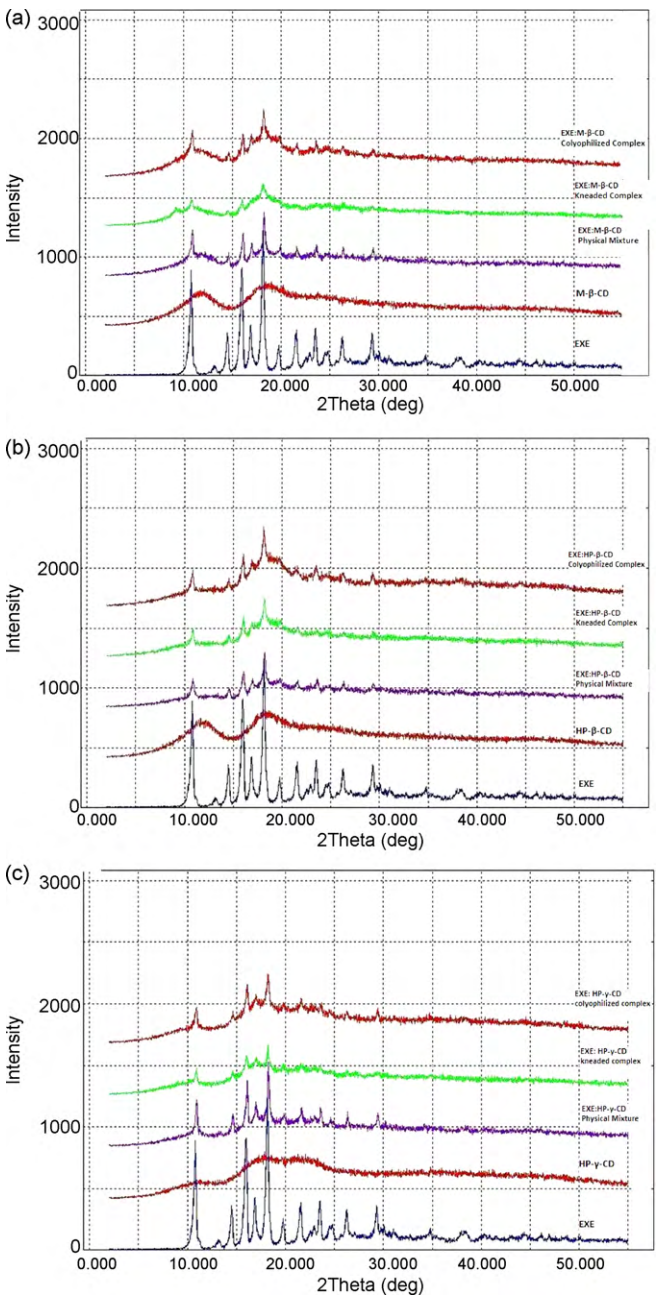

Fig. 4. X-ray diffractograms of EXE, CDs, inclusion complexes and physical mixtures (a: EXE, M-β-CD, EXE:M-β-CD physical mixture, EXE:M-β-CD kneaded complex and EXE:M-β-CD colyophilized complex; b: EXE, HP-β-CD, EXE:HP-β-CD physical mixture, EXE:HP-β-CD kneaded complex and EXE:HP-β-CD colyophilized complex; c: EXE, HP-γ-CD, EXE:HP-γ-CD physical mixture, EXE:HP-γ-CD kneaded complex and EXE:HP-γ-CD colyophilized complex).

changes in microenvironment are known to occur between the free and bound states. During complexation, the chemical environment of some protons changes, and these results in changes in chemical shifts of ^{1}H NMR lines of the protons that are due to shielding or deshielding effects (Bilensoy et al., 2008). The inclusion of an apolar region into the host hydrophobic cavity induces a shielding of the inner protons of the glucose units of CDs, namely, H-3 and H-5, whereas the protons on the exterior of the torus (H-1, H-2 and H-4) are relatively unaffected (Otagiri et al., 1975). For the complexes, H-3 and H-5 protons were evaluated for changes in ^{1}H NMR spectra and the results are given in Table 1. H-5 shifted from 3.86 to 3.63 ppm and H-3 shifted from 3.93 to 3.77 ppm for EXE:M-

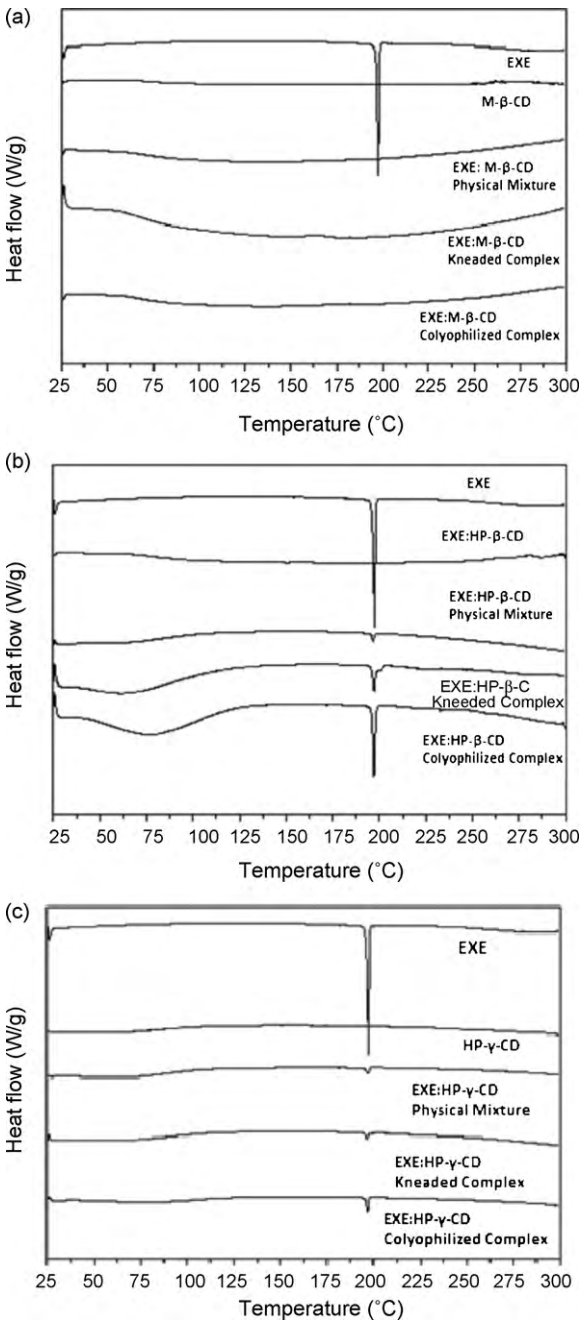

Fig. 5. DSC thermograms of EXE, CDs, inclusion complexes and physical mixtures (a: EXE, M-β-CD, EXE:M-β-CD physical mixture, EXE:M-β-CD kneaded complex and EXE:M-β-CD colyophilized complex; b: EXE, HP-β-CD, EXE:HP-β-CD physical mixture, EXE:HP-β-CD kneaded complex and EXE:HP-β-CD colyophilized complex; c: EXE, HP-γ-CD, EXE:HP-γ-CD physical mixture, EXE:HP-γ-CD kneaded complex and EXE:HP-γ-CD colyophilized complex).

β-CD kneaded complex. Since both H-3 and H-5 proton shifts were changed, it is suggested that the drug has entered the cyclodextrin cavity to form an inclusion complex.

Scanning electron microscopy (SEM) is a qualitative method used to study the structural aspects of raw materials, i.e., CD and drugs or the products obtained by different methods of preparation like physical mixture, solution complexation, coevaporation and others (Duchéne, 1987). SEM photomicrographs of EXE, M-β-CD, HP-β-CD, HP-γ-CD, EXE:CDs inclusion complexes and EXE:CDs physical mixtures were given in Fig. 6. Typical crystal of CDs and EXE were found in physical mixture photomicrographs. These results demonstrate that no complex is formed in the physical mixture of the compounds. The inclusion complex samples were seen as smaller cubic shape that is different form EXE and CD structures. Overall, characterization results indicate the formation of an inclusion complex between EXE and M-β-CD by kneading method is optimum for EXE complexation.

To compare dissolution profiles of different CD derivatives and different preparation methods, distilled water and distilled water containing 0.5% SLS were used as media. 0.5% SLS solution was selected as a dissolution medium because this medium is recommended by FDA for EXE tablets dissolution. Since the inclusion complexes dissolves more than 85% in 15 min, distilled water was used as a medium to compare the differences between the cyclodextrin complexes dissolution profiles. *In vitro* dissolution profiles of EXE and EXE:CD inclusion complexes both in 0.5% SLS solution and distilled water were given in Fig. 7. Each data point represents a mean of six measurements for each product.

In 0.5% SLS medium, the dissolution of EXE powder was incomplete even after 120 min. All the inclusion complexes with all three CDs displayed better dissolution properties with respect to EXE alone, being immediately dispersed and completely dissolved within 10 min. The extent of enhancement of dissolution rate was found to be dependent on the preparation method, since the kneaded products exhibited the highest dissolution rates. The complexes prepared by kneading technique offer dissolution of approximately 100% with 0.5% SLS solution medium and 80% with distilled water medium in 10 min. Complex stability is one of the major factors affecting the dissolution rates because of the influence on free crystalline EXE. The dissolution results are correlated to the complex characterizations and the free crystalline EXE fractions in the complexes.

Since phase solubility studies, solid state characterization techniques and dissolution studies indicate that M-β-CD and kneading method show the optimum results, EXE:M-β-CD kneaded complex was used to develop a tablet formulation as an alternative to commercially available product. The average weight of the tablets was found to be 397 mg (±0.28) and the tablets thickness was 0.27 cm (±0.005). The tablets strength was in the range of 7.9–8.1 kp (±0.12).

Dissolution studies were performed with both EXE:M-β-CD tablets and Aromasin® tablets to compare their dissolution rates. *In vitro* dissolution profiles of EXE:M-β-CD tablets and Aromasin® tablets both in 0.5% SLS solution and in distilled water were given in Fig. 8. Each data point represents a mean of six measurements for each product. The difference factor (f_1) and the similarity factors (f_2) were calculated and given in Table 2.

The dissolution of EXE from the Aromasin® tablets was incomplete even after 120 min both in 0.5% SLS solution and in distilled water, although this commercial formulation contains Polysorbate 80 as a solubility enhancer excipient. EXE:M-β-CD tablets offer a 100% dissolution profile with 0.5% SLS solution medium and 80% with distilled water medium in 10 min, which may be of particular interest for industrial scale preparations because of the low cost and the simple process, which involves less energy, time, and equipment. f_1 and f_2 values were found to be 19.54 and 33.82 for 0.5% SLS solution medium and 31.68 and 32.17 for distilled water medium. Since f_1 value over 15 and f_2 value between 0 and 50 suggests the two dissolution profiles different, the dissolution profiles of EXE:M-β-CD tablets and Aromasin® tablets were demonstrated to be statistically different.

Cyclodextrins have been suggested to act as drug carrier to the gastrointestinal membrane and were reported to enhance penetration of drugs in the intestine. CDs act as carrier by masking physicochemical properties of hydrophobic drugs in solution and delivering these drugs in a microconcentration gradient to the cell membrane where they partition in the membrane (Rajewski

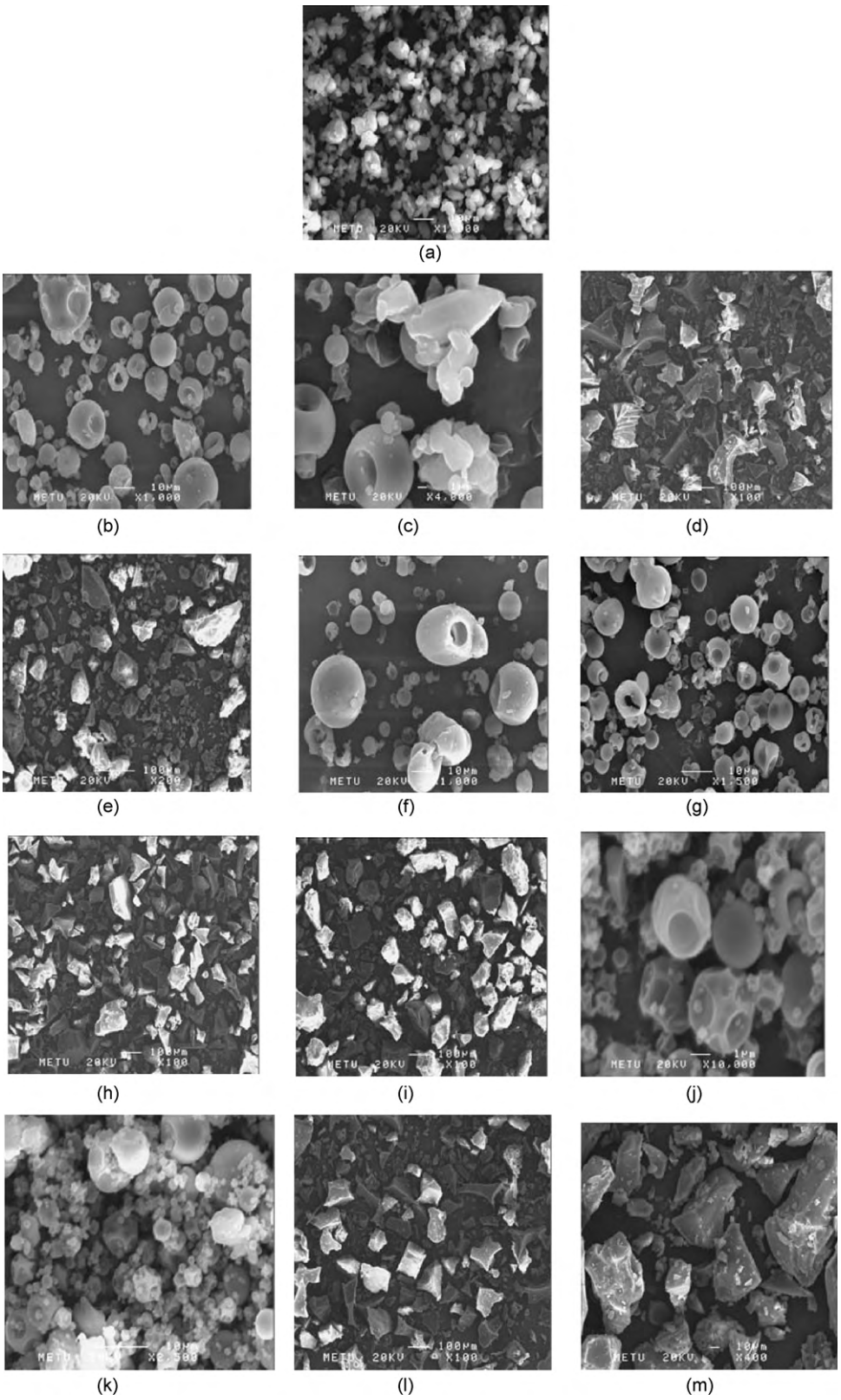

Fig. 6. SEM photomicrographs of EXE, CDs, inclusion complexes and physical mixtures (a: EXE; b: M-β-CD; c: EXE:M-β-CD physical mixture; d: EXE:M-β-CD kneaded complex; e: EXE:M-β-CD colyophilized complex; f: HP-β-CD; g: EXE:HP-β-CD physical mixture; h: EXE:HP-β-CD kneaded complex; i: EXE:HP-β-CD colyophilized complex; j: HP-γ-CD; k: EXE:HP-γ-CD physical mixture; l: EXE:HP-γ-CD kneaded complex; m: EXE:HP-γ-CD colyophilized complex).

and Stella, 1996; Zuo et al., 2000). Although the detailed mechanism of CDs action in enhancing the transcellular route has not yet been clarified, it seems likely that the regular arrangement of lipid molecules, which constitute the cell membrane is perturbed by the interaction of membrane lipids and CDs (Nakanishi et al., 1992). Different cyclodextrins used in this study have shown increased solubility and dissolution rate. Therefore, it was crucial to investigate whether complexation enhances permeability (and thereby

Table 1
Chemical shift values (ppm) of M-β-CD, HP-β-CD and HP-γ-CD protons prior to and following complexation to EXE.

	M-β-CD	EXE:M-β-CD physical mixture	EXE:M-β-CD kneaded complex	EXE:M-β-CD colyophilized complex
H3	3.93	3.82	3.77	3.82
H5	3.86	3.70	3.63	3.67
	HP-β-CD	EXE:HP-β-CD physical mixture	EXE:HP-β-CD kneaded complex	EXE:HP-β-CD colyophilized complex
H3	3.82	3.96	3.97	3.95
H5	3.82	3.83	3.85	3.83
	HP-γ-CD	EXE:HP-γ-CD physical mixture	EXE:HP-γ-CD kneaded complex	EXE:HP-γ-CD colyophilized complex
H3	3.98	3.95	3.93	3.95
H5	3.87	3.82	3.77	3.82

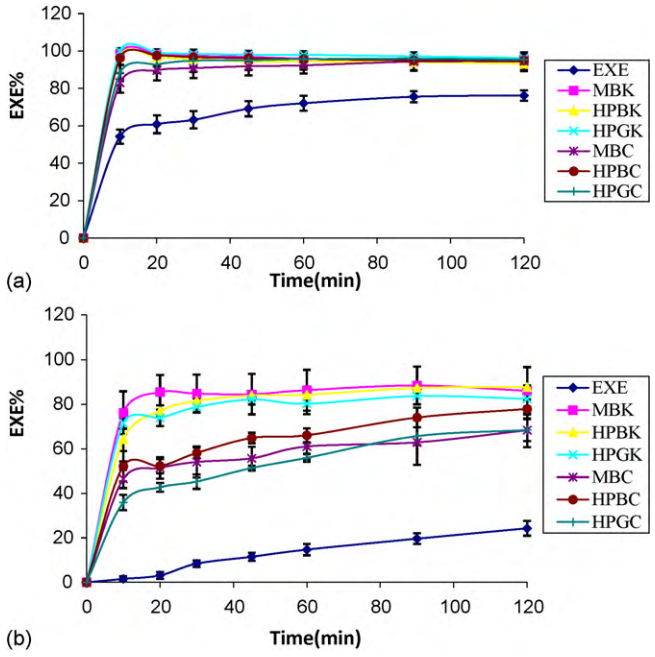

Fig. 7. Mean dissolution profiles of EXE and inclusion complexes ($n = 6$, ±SD, MBK, EXE:M-β-CD kneaded complex; MBC, EXE:M-β-CD colyophilized complex; HPBK, EXE:HP-β-CD kneaded complex; HPBC, EXE:HP-β-CD colyophilized complex; HPGK, EXE:HP-γ-CD kneaded complex; HPGC, EXE:HP-γ-CD colyophilized complex) (a: medium 0.5% SLS solution and b: medium distilled water).

bioavailability) of EXE against Caco-2 cells. The apical to basolateral permeability of EXE alone or EXE:CD inclusion complexes are shown in Table 3 and Fig. 9. Permeability co-efficient (P_{app}) for EXE:HP-γ-CD is significantly higher ($P < 0.05$) than those of EXE alone, EXE:M-β-CD and EXE:HP-β-CD complexes. All complexes resulted in at least 2-fold increase in permeability constants. As can be seen in Table 3, P_{app} for EXE:M-β-CD is 2.41 times, EXE:HP-β-CD is 2.05 times and EXE:HP-γ-CD is 3.30 times higher than the P_{app} for EXE alone are also statistically significant ($P < 0.05$).

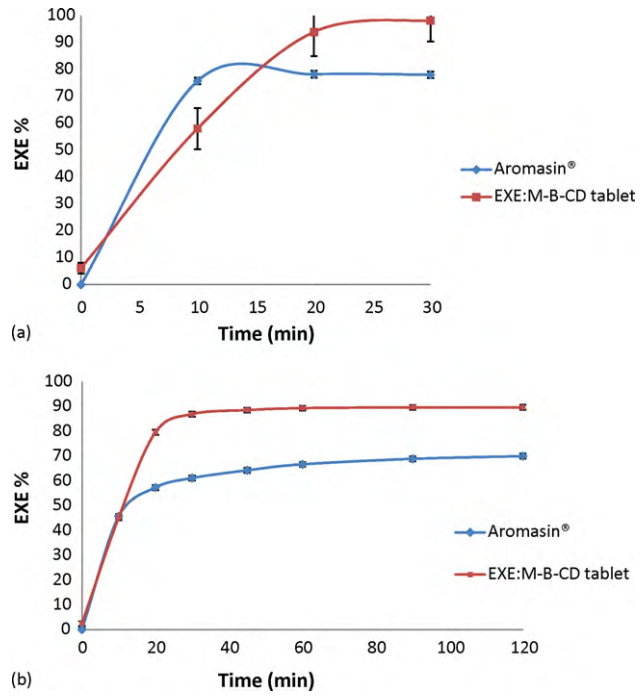

Fig. 8. Mean dissolution profiles of EXE:M-β-CD tablets and Aromasin® tablets ($n = 6$, ±SD) (a: in 0.5% SLS solution in water and b: in distilled water).

Table 2
f_1 and f_2 statistics for EXE:M-β-CD tablets and Aromasin® tablets ($n = 6$).

	Aromasin® tablet–EXE:M-β-CD tablet	
	Medium: 0.5% SLS solution	Medium: distilled water
f_1	19.54	31.68
f_2	33.82	32.17

Table 3
Increase in apparent permeability (P_{app}) values across Caco-2 cells of EXE or EXE complexes.

Sample	P_{app} (mean ± SD, $n = 3$) cm/s	×Exemestane
EXE	$5.52\ (\pm 1.67) \times 10^{-6}$	1
EXE-M-β-CD	$13.3\ (\pm 0.25) \times 10^{-6}$	2.41
EXE-HP-β-CD	$11.3\ (\pm 0.22) \times 10^{-6}$	2.05
EXE-HP-γ-CD	$18.2\ (\pm 0.15) \times 10^{-6}$	3.30

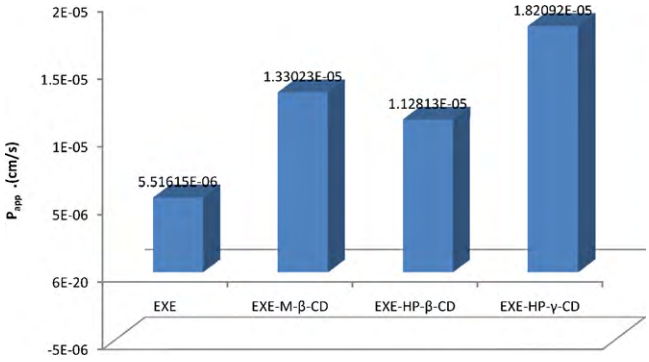

Fig. 9. Apparent permeability (P_{app}) values across Caco-2 cells of EXE or EXE complexes.

Although the detailed mechanism of CD action in enhancing permeability has not yet been clarified, it seems likely that the regular arrangement of lipid molecules, which constitute the cell membrane is perturbed by the interaction of membrane lipids and CDs. It is also reported that CDs increase permeability of Caco-2 cell monolayers by displacing specific claudins from cholesterol rich domains associated with tight junctions. Thus, cyclodextrin permeability enhancing properties depend on their physicochemical properties such as molecular weight and cavity size (Nakanishi et al., 1992; Lambert et al., 2007). HP-γ-CD which has a larger inner cavity might have shown better interaction with membrane lipids.

4. Conclusions

In this study, EXE:CD inclusion complexes were prepared in order to improve the solubility and dissolution rate and enhance the intestinal permeability of EXE and a new oral tablet formulation was designed using EXE:M-β-CD inclusion complex and compared with tablets in the pharmaceutical market. In the light of data obtained in this study, it can be concluded that an effective oral tablet formulation of EXE in terms of increased aqueous solubility of the drug, higher dissolution rate and increased intestinal permeation by complexation to modified cyclodextrins, methyl-β-cyclodextrin and kneading method in particular, can be achieved. Properties of EXE, which is reported in the literature as a BCS Class IV drug, were altered by inclusion complexation such that EXE can be suggested to be behaving like Class I or Class II when complexed to cyclodextrins. This phenomenon requires further *in vivo* bioavailability studies to be better elucidated. Thus this is a promising approach to improve the poor oral bioavailability of EXE which is commonly used for long term oral administration in postmenopausal breast cancer chemotherapy in clinics.

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