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RESEARCH ARTICLE

Evaluation of cefpodoxime proxetil complex with hydroxypropyl-β-cyclodextrin in the presence of a water soluble polymer: Characterization and permeability studies

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Evaluation of Cefpodoxime proxetil Komplex with Hydroxypropyl-β-cyclodextrin in the Presence of a Water Soluble Polymer: Characterization and Permeability Studies

Summary

This study aims to prove the complexation of cefpodoxime proxetil (CP) by hydroxypropyl-β- cyclodextrin (HP-β-CD) in the presence of sodium carboxymethyl cellulose (Na CMC), and investigates the permeability of CP in the complexes. The CP–HP-β-CD complex was prepared by kneading method and was characterized by Scanning electron microscope (SEM), Fourier transform infrared spectroscopy (FTIR) and Differential scanning calorimetry (DSC). The solubility method was used to investigate the effect of HP-β-CD and Na CMC on the solubility of CP. Furthermore, permeability studies were performed with Caco-2 cells. It was observed in all formulations that complexation with HP-β-CD lead to increase of the CP solubility and permeability, compared to the CP bulk. It is evident from the results that complexation with HP-β-CD in the presence of Na CMC is promising way to prepare more efficient complex formulation with improved solubility and permeability.

Key Words: *Cefpodoxime proxetil, cyclodextrin, sodium carboxymethyl cellulose, permeability, complex*

Hidroksi propil-β-siklodekstrin ile Sefpodoksim proksetil Kompleksinin Suda Çözünen Polimer Varlığında Değerlendirilmesi: Karakterizasyonu ve Geçirgenlik Çalışmaları

Özet

Bu çalışmada sefpodoksim proksetilin (CP) sodyum karboksi metil selüloz (Na CMC) varlığında hidroksi propil beta siklodekstrinle (HP-β-CD) kompleksinin incelenmesi ve permeabilitesinin değerlendirilmesi amaçlanmıştır. CP-HP- β -CD kompleksi örgü yöntemiyle hazırlanmış; Elektron mikroskop görüntüleme sistemi (SEM), Fourier transform infrared sepktroskopisi (FTIR) ve Diferansiyel görüntüleme kalorimetrisi (DSC) yöntemiyle karakterize edilmiştir. CP nin çözünürlüğü üzerine Na CMC ve HP- β -CD etkisi incelenmiştir. Ayrıca permeabilite çalışmaları, Caco-2 hücreleri kullanılarak yapılmıştır. Sonuç olarak tüm formülasyonlar CP toz haliyle karşılaştırıldığında, HP- β -CD'in CP'nin çözünürlüğünü ve permeabilitesini arttırdığı gözlenmiştir. Na CMC varlığında hazırlanan CP-HP- β -CD kompleksleri permeabilite ve çözünürlük açısından değerlendirildiğinde daha etkili kompleks formülasyonu hazırlanması için istenen bir yol olduğu elde edilen sonuçlardan açıkça görülmektedir.

Anahtar Kelimeler: *Sefpodoksim proksetil, siklodekstrin, sodyum karboksi metil selüloz, permeabilite, kompleks*

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INTRODUCTION

Cefpodoxime proxetil (CP) is a third generation oral cephalosporin, which is of class β-lactam antibiotics, a broad spectrum activity against many gram positive and gram negative bacteria. CP is used for the treatment of a great variety of infections like skin, respiratory, urinary tract, and systemic infections (1, 2, 3, 4, 5). Several studies have been reported about the oral absorption and bioavailability of CP. The absolute bioavailability of CP after the application as a single dose of 130 mg (equivalent to 100 mg of cefpodoxime) in humans is only about 50% (3, 4). This low bioavailability is mainly attributed to the degradation of its ester side chain by cholinesterases, which are present in the intestinal lumen (5). The poor CP water solubility of about 400 µg/mL is responsible for its low bioavailability, which can cause an insufficient intestinal absorption (6, 7).

The poor water solubility of drugs like CP has been improved by several techniques like a physical modification of drug molecule, the use of excipients as the development of novel dosage forms (8). The Biopharmaceutic Classification System (BCS) is a scientific framework for classifying drug substances based on their aqueous solubility and their intestinal permeability. The solubility and permeability properties are the most important parameters affecting the bioavailability. CP is a BCS Class IV drug, with a poor aqueous solubility and a low permeability (9).

Cyclodextrins (CDs) have the ability to interact with poor water soluble drugs and improve their solubility (10, 11). It is also reported in the literature that CD complexation enhances oral bioavailability of poor soluble drugs (11, 12, 13). Among the CDs, hydroxypropyl-β-cyclodextrin (HP-β-CD) can be used for an oral or intravenous administration (14, 15). In addition, to increase the solubility and the bioavailability of drugs, HP-β-CD was much less toxic than the natural β-CD (16). Many reports showed the ability of water soluble polymers to enhance both the solubility of a complex and the CD complexation efficiency (17, 18, 19). Therefore, complexation with HP-β-CD in the presence of water soluble polymer is a possible way to prepare better soluble and permeable oral product for the application of CP.

Carboxymethyl cellulose (CMC), an important ionic derivative of cellulose, is usually used as its sodium salt (Na CMC). Na CMC is widely applied in several industrial sectors including the food, paper, paint, pharmaceutical, cosmetics and mineral processing. Recently, due to the good biocompatibility, Na CMC has been drawing attention as a representative water-soluble polysaccharide in many research fields, such as drug delivery and tissue engineering (20, 21, 22, 23).

The aim of this study was to improve the water solubility and the permeability of CP, which consequently leads to an enhanced oral bioavailability of CP. To achieve this goal, the complexes were prepared by kneading method and were characterized by using HP- β-CD with DSC and FTIR spectroscopy (17, 18). Furthermore, the effect of water soluble polymer such as Na CMC, on the complexation -efficiency of CP with HP-β-CD and the permeability of CP after complexation were investigated. All complexes were comparatively evaluated in terms of increasing the solubility and the in vitro permeation through human colon adenocarcinoma cell line (Caco-2 cell).

MATERIALS AND METHODS

Materials

CP was a kind gift from Nobel Drug Company (Istanbul, Turkey). Hydroxypropyl-β-cyclodextrin (HP-β-CD), Cavasol W7HP, was supplied from Wacker Chemie, Netherland. Na CMC was purchased from Sigma Aldrich. High performance liquid chromatography (HPLC) grade acetonitril and ammonium acetate were purchased from Merck. Cell culture reagents and supplies were obtained from GIBCO Invitrogen Co. (United Kingdom). Caco-2 cell line, used for the permeability studies, was obtained from American Type Culture Collection (ATCC).

Methods

Phase solubility studies

Phase solubility studies were performed in the absence and presence of Na-CMC. Aqueous solutions were prepared containing HP-β-CD (0, 5, 10, 20, 30% w/v). An excess CP was added to solutions and stirred at room temperature until equilibrium was reached (7 days). After this equilibrium, the suspensions were

filtered through a 0.45 µm polycarbonate membrane filter and the solutions were analyzed by HPLC (Agilent 1100, Germany). Phase solubility diagrams were prepared by plotting the HP-β-CD concentration versus the solubilized drug concentration. The apparent stability constants (Ks) of the inclusion complex were determined from the phase solubility diagrams according to Higuchi and Connors (1965) (24). As the slope of these diagrams was <1, it was assumed that a 1:1 stoichiometric complex was formed. Ks were determined from Eq. 1.

$$Ks = \frac{slope}{(1 - slope) \times Sw}$$ **Equation 1**

Sw is the intrinsic solubility (the solubility of CP in the different ratio of HP-β-CD solution (0, 5, 10, 20, 30% w/v)).

For the phase solubility studies in the presence of Na-CMC, 0, 5, 10, 20, 30% (w/v) solutions of HP- β-CD and 0, 0.1, 0.25, 0.5% (w/v) of Na-CMC in distilled water was added to separate vials. After that, the same procedure was applied.

Preparation of CP- HP-β-CD complex in the absence and presence of Na CMC

According to the literature, the inclusion complexes are prepared by two alternative techniques; kneading and colyophilization methods (13). Advantages of the kneading method are simplicity and low cost. In this study, we used the kneading method for preparation of the complexes. Briefly, CP and HP-β-CD (in a 1:1 molar ratio) were mixed by adding ethanol and distilled water as a mixture (1:1 of v/v ratio) until obtaining homogeneous paste and dried at 40°C in an oven for 4 h. For the preparation of CP-HP β-CD complex in the presence of Na-CMC, CP and HP-β-CD (in a 1:1 molar ratio) were mixed by adding ethanol and Na-CMC solution (0.1% w/v) as a mixture (1:1 of v/v ratio) until obtaining a homogeneous paste, dried in an oven at 40°C for 4 h.

CHARACTERIZATION OF COMPLEXES

Fourier transform infrared (FT-IR) spectroscopy

Fourier transform infrared (FT-IR) spectra of CP (3-4 mg), HP-β-CD (3-4 mg), Na CMC (3-4 mg) and CP-HP-β-CD complexes in the absence and presence of Na CMC with potassium borumure (3-4 mg) were taken with a Perkin –Elmer spectrum 100 between 600 and 4000 cm^{-1}.

Differential scanning calorimetry (DSC)

Differential scanning calorimetry analyses were performed with CP, HP-β-CD, Na CMC and CP-HP-β-CD complexes in the absence and presence of Na CMC with a Perkin Emler DSC 8000. Samples weighing approximately 3-4 mg were heated in hermetically sealed aluminum pans at a rate of 10°C/min, between 25 and 250°C.

Scanning electron microscopy (SEM)

Scanning electron images were taken using a Philips XL-30 SEM. The surface characteristics of CP (3-4 mg), HP-β-CD (3-4 mg), Na CMC (3-4 mg) and CP-HP-β-CD complexes in the absence and presence of Na CMC (3-4 mg) were investigated. The instrument settings were as follows: 4-kV acceleration potential, 7–8 mm working distance, and condenser lens setting of 25. The samples were coated with about 10 nm of gold/palladium alloy using a Hummer 6.2 sputter coater. SEM images of samples were obtained at different magnifications.

High performance liquid chromatography (HPLC) analysis of cefpodoxime proxetil

The HPLC method was developed and validated for detecting quantities of CP from the phase solubility and permeability studies. An HPLC system (Agilent 1100, Germany) equipped with a UV-Vis spectrophotometric detector was used for this purpose. C_{18} column (250 mm × 4mm, 5 µm, LiChroCART, Germany) was used in this study. The HPLC method employed acetonitril and ammonium acetate buffer (0.025 molar) at pH 4.5 as mobile phase with a ratio of 35:65, pumped at a flow rate of 1 mL/min, and the analysis were carried out at 30°C with detection at 235 nm. The injection volume was 50 µL. All samples were filtrated through a membrane filter (0.2 µm Nylon, Millipore Millex-GN), before injection. Retention time was 4.2 minutes and the analysis time was 10 minutes. The peak area correlated linearly with CP concentration in the range of 5–200 µg/mL

with the lowest detection limit at 0.5 µg/mL, and the average correlation coefficient was 0.995. LOD and LOQ were found as 0.166 and 0.5 µg/mL, respectively.

Caco-2 cell cultures

The Caco-2 cells were cultured in Dulbecco Modified Eagle Medium (DMEM) from passage number 32 to passage number 67. Cell monolayers were prepared by seeding 4×10^{5} cells/one well on six wells with transwell insert filter. Cell culture was maintained at 37°C under 90% humidity and 5% CO_2. Monolayers were used 19–22 days after seeding. The integrity of each cell monolayer was checked by measuring its transepithelial electrical resistance (TEER) with an epithelial voltameter (EVOM, World Precision Instrument, Sarasota, FL, USA), before and after the experiments. The TEER value was measured from the following Equation 2:

$$TEER = (R_{monolayer} - R_{blank}) \times A \quad \textbf{Equation 2}$$

$R_{monolayer}$ is the resistance of the cell monolayer along with the filter membrane, R_{blank} is the resistance of the filter membrane and A is the surface area of the membrane (4.7 cm^2 in six well plates) (25).

Permeability of cefpodoxime proxetil and its complexes

The in vitro permeability study was developed in Caco–2 cell monolayers, grown in transwell inserts with a collagen coated polycarbonate membranes with a pore size of 0.4 µm and a surface area of 4.7 cm^2 in cluster. The cells were maintained at 37°C in an atmosphere as described above. The medium was replaced every second day for 3 weeks. For the experiments with Caco–2 cell monolayers, CP and CP-HP-β-CD complexes in the absence and presence of Na-CMC were used.

Culture medium was replaced from each well by 1 mL and 1.5 mL Hank's Balanced Salt Solutions (HBSS, pH = 7) in the apical and basolateral side of the well and the cell monolayers were subsequently equilibrated for 30 min at 37°C. The CP or CP-HP-β-CD complexes were applied to Caco-2 cell with a concentration of 200 µg/mL. CP and CP-HP-β-CD complex solutions in HBSS were added to the apical side (A, 2.2 mL) from the apical to the basolateral direction (A →B) or to the basolateral side (B, 3.2 mL) from the basolateral to the apical direction (B → A) (pH = 7). The six-well plates containing the cell monolayer were placed into an orbital environmental shaker, kept at a constant temperature (37°C) and an agitation rate of 50 rpm for the duration of the transport experiments (2 h). Serial samples of 200 µL each were taken at 30-min intervals from the basolateral to apical direction and analyzed by HPLC.

Data analysis

Apparent permeability values (P_{app}) for each side, efflux ratio and recovery % (mass balance) were calculated according to the following equations:

$$P_{app} = \frac{dQ}{dt}\ \frac{1}{A \times C_0 \times 60} \quad \textbf{Equation 3}$$

$$Efflux\ ratio = \frac{P_{app}\ (B \to A)}{P_{app}\ (A \to B)} \quad \textbf{Equation 4}$$

$$Recovery\ \% = \frac{(C_{fd} \times V_d) + (C_{fd} \times V_d)}{C_{od} \times V_d} \quad \textbf{Equation 5}$$

Where P_{app} is the apparent permeability (cm/s), dQ/dt is the permeability rate, A is the diffusion area of monolayers (cm^2), and C_0 is the initial concentration of the drug in the donor compartment. Efflux ratio was expressed as the quotient of P_{app} (B →A) to P_{app} (A →B) (26, 27). A recovery (mass balance) calculation was also performed to determine if the accumulation or the metabolism of the solute or adsorption to the apparatus occured. Where C_{od} and C_{fd} are the initial and final concentrations of the compound in the donor compartment, respectively; C_{fa} is the final concentration of acceptor compartment; V_d and V_a are the volumes of the solutions in the donor and acceptor compartments.

Statistical data analyses

Statistical analyses were performed using one way analysis of variance (ANOVA) to evaluate the differences between CP and CP-HP-β-CD complex. Data were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSIONS

Phase solubility studies

The effect of HP-β-CD on the solubility of CP in the presence and the absence of the water-soluble polymer Na CMC was investigated. The determination of the phase solubility diagram is a widely accepted method for the evaluation of the effect of CD complexation on the drug solubility. The drug/CD complex (1:1) is the most common type of association where a single drug molecule is included in the cavity of one CD molecule, with a stability constant $K_{1:1}$ for the equilibrium between the free and the associated species (28). The phase solubility diagrams are presented in Fig. 1. The

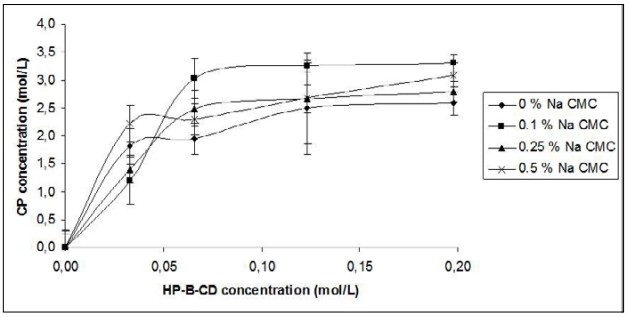


Figure 1. Phase solubility diagram of CP with HP-β-CD in the absence and presence of Na CMC in distilled water

stability constants ($K_{1:1}$) were calculated from the initial straight-line part of the solubility curves and were about 430.8 mol^{-1} in the absence and 730.4 mol^{-1} in the presence of the 0.1% Na CMC (w/v), respectively. However, increased Na CMC concentration of 0.25% and 0.5% (w/v), stability constant is similar to that observed in 0.1% Na CMC ($p < 0.05$) (Table 1). As expected, the solubility of CP was increased with increasing the concentration of HP-β-CD. Furthermore there is a linear relationship between CP solubility and HP-β-CD concentration in the presence and absence of Na CMC. In addition, CP solubility was increased 1.3-fold after the addition of Na CMC at each concentration point of HP-β-CD. In a previously conducted study, it was reported that the solubility of naproxen increased when a water soluble polymer like hydroxypropylmethylcellulose (HPMC) was combined with CD derivatives like sulfobutyl ether β-cyclodextrin (SBE7-β-CD). This synergistic improvement of the drug solubility was probably due to the ability of the water soluble polymer increasing the concentration of free naproxen available in solution, which interacts with CD derivatives (17). Katzhendler et al. (29) studied the complexation of carbamazepine with CD in the presence of HPMC, then assumed that the increasing solubility of carbamazepine was caused by the interaction between HPMC and carbamazepine in solution, and considered this was caused by the hydrogen bonding. This experiment suggests the possible interaction between the drug and water soluble polymer during the complexation process.

Table 1. The stability constant values in the absence and presence of Na CMC for CP-HP-B-CD complexes

Na CMC % (w/v)	K1:1
0	430.8
0.1	730.4
0.25	722.8
0.5	731.6

FT-IR spectroscopy, DSC and SEM studies

To prove the complex formulation FT-IR spectroscopy, differential scanning calorimetry and SEM analysis were performed. FT-IR spectra of CP, HP-β-CD, Na CMC and CP-HP-β-CD complexes in the absence and presence of Na CMC are presented in Fig. 2 and 3. FT-IR spectra of the complexes indicated the

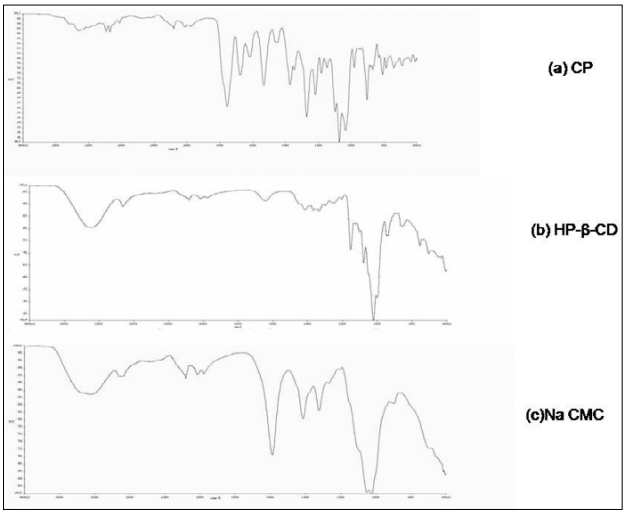


Figure 2. FTIR spectrum of CP, HP-β-CD and Na CMC

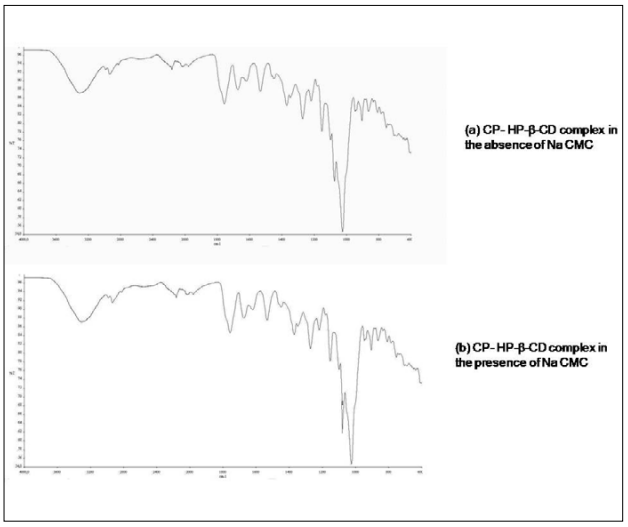


Figure 3. FTIR spectrum of CP-HP-β-CD complexes in the absence and presence of Na CMC

disappearance of typical bands of CP like C = O stretch peak at 1756 cm^{-1} and N-H strech peak at 1325 cm^{-1}. C-H stretching regions are present in both the CP and the CD structures, thus the disappearance of C = O stretch peak was not expected upon complexation. The O-H stretching bands are typical for CDs, but any shifts of these bands might indicate the formation of hydrogen bonds between CP and HP-β-CD. These results showed appreciable shifts and variation in intensity of the characteristic CP bands, evidencing the chemical interactions between the CP and HP-β-CD due to inclusion of the drug in the CD cavity. Furthermore, the FT-IR spectra of CP changed in the CP-HP-β-CD complex in combination with Na CMC.

The DSC profiles of pure CP, HP-β-CD, Na CMC and CP-HP-β-CD complexes in the absence and presence of Na CMC are shown in between Fig. 4 and 5. The DSC result of pure CP showed no endothermic peak between 25 and 250°C, while the thermogram of HP-β-CD and Na CMC shows an endothermic band ranging between 160 and 200°C. The thermogram of CP changed in the CP: HP-β-CD complexes (in the absence and presence of Na CMC) (Fig. 4 and 5). These thermal behaviour changes indicate that the evidence of the inclusion complex through molecular interactions between the CP, HP-β-CD and Na CMC.

SEM pictures of CP, HP-β-CD, Na CMC and CP-HP-β-CD complexes in the absence and presence of Na CMC were given in Fig. 6. The complex products presented a different morphology according to CP, HP-β-CD and Na CMC. When the HP-β-CD is kneaded with CP, the cavities on the HP-β-CD are filled by molecules of CP. Overall, characterization results indicate the formation of an inclusion complex between CP and HP-β-CD by kneading method is suitable for CP complexation, with and without Na CMC.

Permeability of cefpodoxime proxetil and its complexes

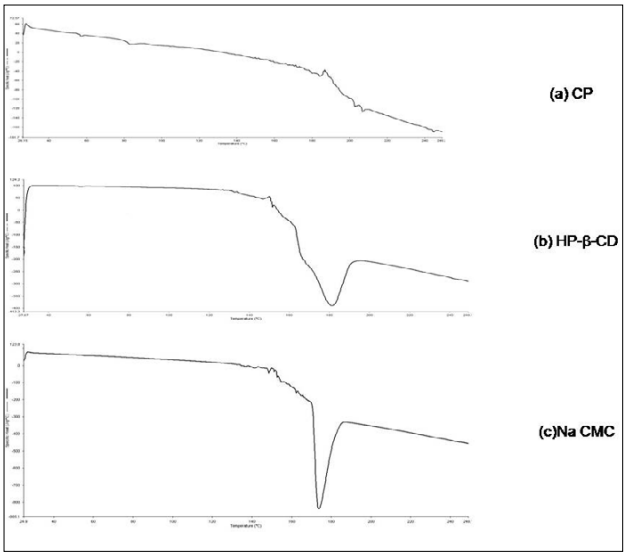


Figure 4. DSC spectra of CP, HP-β-CD and Na CMC

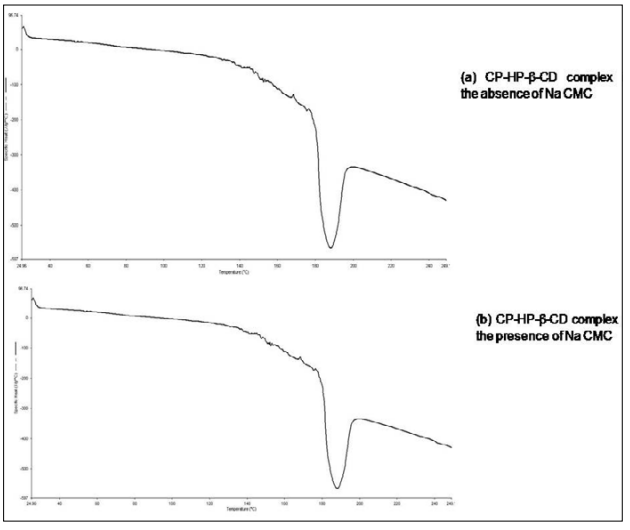


Figure 5. DSC spectra of CP-HP-β-CD complexes in the absence and presence of Na CMC

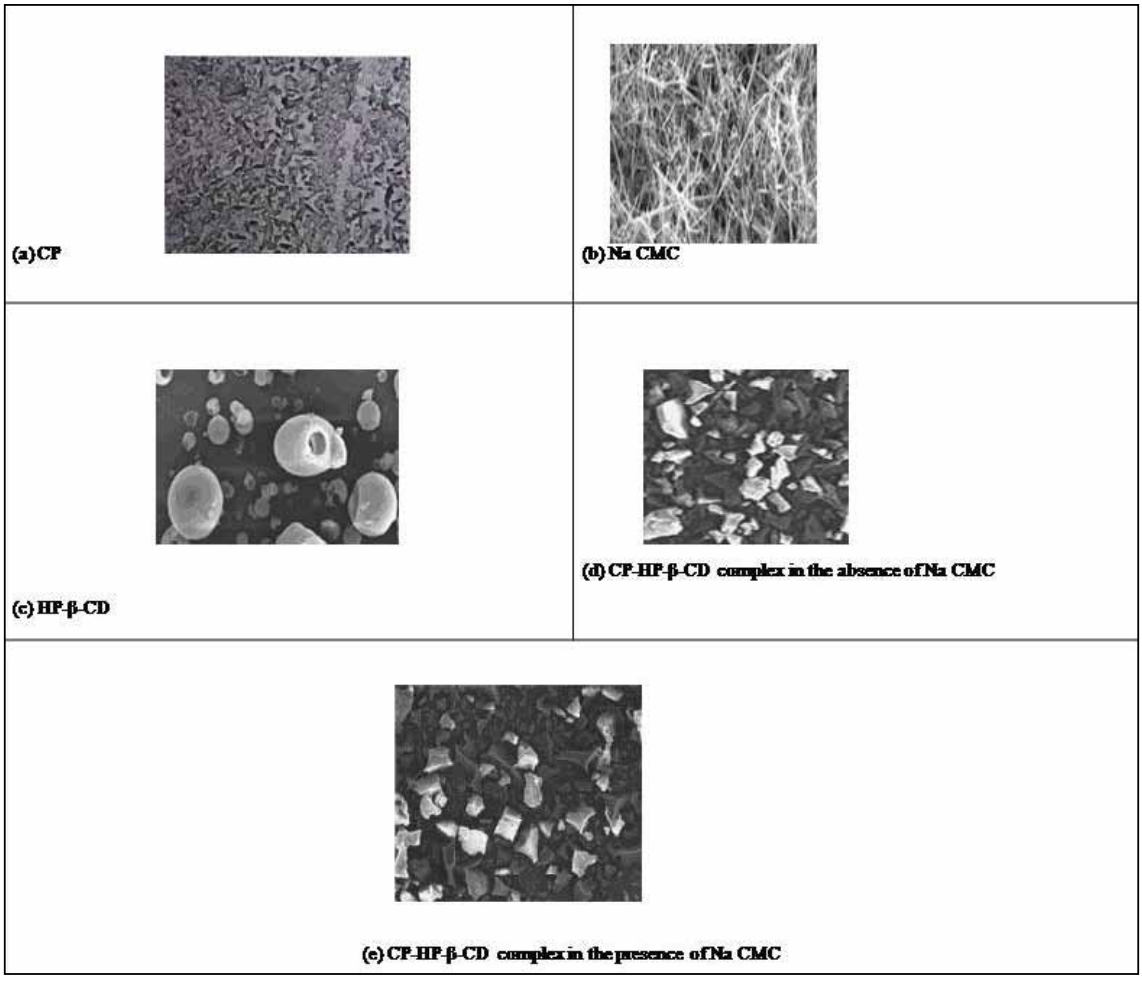
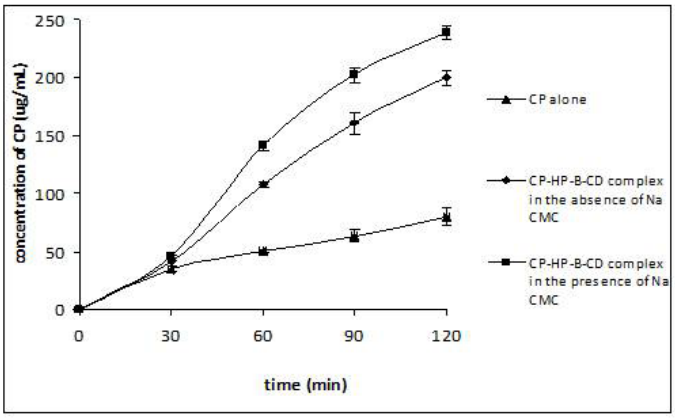


Figure 6. SEM micrographs of CP, Na CMC, HP-B-CD and CP-HP-B-CD complexes in the absence and presence of Na CMC

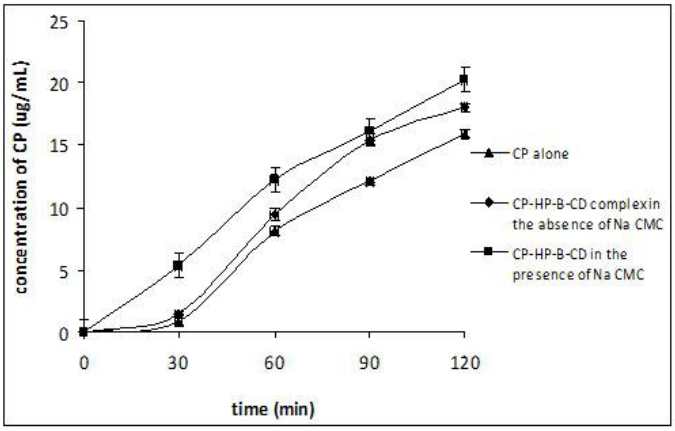
Caco-2 cell permeability test is considered as a reliable tool for screening the transport efficiency of new drugs and formulations (30). In this study, the permeability of CP across Caco-2 cells using CP bulk and CP-HP-β-CD complexes in the absence and presence of Na CMC were evaluated. Figure 7 a and b show the permeated concentration of CP from complexes in the absence and presence of Na CMC and bulk across the Caco–2 cell monolayers. The permeability coefficient values (P_{app} (A→B) and P_{app} (B→A)), efflux ratio and percentage recovery (recovery%) of CP alone and in CD complexes are shown in Table 2.

Generally, substances with an apparent permeability coefficient (P_{app}) of less than 1×10^{-6} cm/s are classified as low permeability substances. Medium permeability substances have P_{app} values between 1×10^{-6} and 1×10^{-5} cm/s and high permeability substances exhibit apparent permeability coefficients of $> 1 \times 10^{-5}$ cm/s (31).

CDs 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 disperse in the membrane (32, 33). Although the detailed mechanism of CDs action in enhancing the transcellular route has not yet been clarified, the regular arrangement of lipid molecules, which constitute the cell membrane, is probably interfered by the interaction of membrane lipids with CDs (34). HP-β-CD used in this study has shown an increased solubility. Therefore, it was



(a) Permeated concentration of CP apical to basolateral side for CP-HP-B complexes in the absence and presen Na CMC



(b) Permeated concentration of CI from basolateral to apical side fo CP-HP-B-CD complexes in th absence and presence of Na CMC

Figure 7. Permeated concentration of CP from CP-HP-B-CD complexes in the absence and presence of Na CMC across Caco–2 cell. (a) Permeated concentration of CP from apical to basolateral side for CP-HP-B-CD complexes in the absence and presence of Na CMC (b) Permeated concentration of CP from basolateral to apical side for CP-HP-B-CD complexes in the absence and presence of Na CMC. Data are expressed as means ±SD of three experiments

crucial to investigate whether the complexation enhanced permeability (and thereby bioavailability) of CP against Caco–2 cells. According to permeability results, the permeability values for the apical to basolateral direction (P_{app} (A→B)) were significantly higher than the permeability value for the basolateral to apical direction (P_{app} (B→A)) ($p < 0.05$). In addition, the permeated concentration of CP across Caco-2 cell monolayers in the apical to basolateral (A→B) direction was much higher than that in the basolateral to apical (B→A) direction for all formulations (Fig. 7 a and b). The permeability

Table 2. The permeability values, efflux ratios and recovery % of CP alone, CP-HP-B-CD complexes in the absence and presence of Na CMC with ±SD. P_{app} (A→B), permeability value for A → B direction. P_{app} (B→A), permeability value for B → A direction

Formulations	Papp (A→B) (cm/s)	Papp (B→A) (cm/s)	Efflux ratio	Recovery %
1	0.9×10-6 ±0.0035	2.28×10-7 ±0.0025	0.25 ±0.04	1.13 ±0.09
2	2.74×10-5 ±0.0021	2.64×10-6 ±0.0040	0.096 ±0.012	1.44 ±0.14
3	3.35×10-5 ±0.0048	2.71×10-6 ±0.0036	0.081 ±0.023	1.54 ±0.21

*Each value is the mean ±SD of three experiments. 1- CP alone, 2- CP-HP-B-CD complexes in the absence of Na CMC, 3- CP-HP-B-CD complexes in the presence of Na CMC

value (P_{app} (A→B)) was found 0.9× 10^{-6} ±0.0035 cm/s for CP alone. However, the permeability value (P_{app} (A→B)) was higher than 1 × 10^{-5} cm/s for CP-HP-β-CD complexes in the absence and presence of Na CMC. Based on these values, it can be concluded that CP is low permeable according the Caco-2 cell monolayer. When P_{app} (A→B) values were compared between different formulations, P_{app} (A→B) value of CP- HP-β-CD complexes (P_{app} (A→B) = 2.74×10^{-5} ±0.0021 cm/s for CP-HP-β-CD complexes in the absence of Na CMC and about P_{app} (A→B) = 3.35×10^{-5} ±0.0048 cm/s for CP-HP-β-CD complexes in the presence of Na CMC) was higher than CP alone (P_{app} (A→B) = 0.9× 10^{-6} ±0.0035). The CP-HP-β-CD complex in the presence of Na CMC shows a higher permeability of CP compared to the CP-HP-β-CD complex in the absence of Na CMC and to CP alone, respectively.

The effects of HP-β-CD and Na CMC on the efflux of CP across Caco–2 cell monolayers were investigated. The efflux ratio of CP alone was about 0.25 ±0.04. When the permeability studies were carried out with the CP-HP-β-CD complex in the absence and presence of Na CMC, the efflux ratio of CP decreased to 0.096 ±0.012 and 0.081 ±0.023 for CP-HP-β-CD complex in the absence and presence of Na CMC, respectively (Table 2). Wang et al. (2009) showed that there was not any transporter involved in the permeability process of drug compound across Caco–2 cell monolayer, when efflux ratio of drug compound was less than 2 (26). In this study, the results indicate the possibility of absorptive transporters to be involved in CP transport. Gundogdu et al. (2011) revealed that at different fexofenadine concentrations, the P_{app} (B→A) values were higher than P_{app} (A→B) values confirming the presence of an efflux transporter for fexofenadine (27). However, CP exhibited a higher permeability value in the A →B direction when compared to B →A direction indicating that CP is likely not a substrate. The percentage recovery (recovery%) of CP alone, CP-HP-β-CD complex in the absence and presence of Na CMC was found as 1.13 ±0.09, 1.44 ±0.14 and 1.54 ±0.21, respectively (Table 2). The permeability results for all formulations showed a recovery rate under 30%. Hellinger et al. (2010) studied on Caco–2 cell line the permeability with different compounds (35). They found a recovery rate under 30% for compounds, except of loperamid (50-60%). According to their results, they reported that the tested compounds were permeated across Caco–2 cell, but were not present in the cells and on the surface of the cells in the exception of loperamid (35). In this study, recovery results revealed that a substantial amount of CP in all formulations can not entrap into the Caco–2 cell monolayers. Moreover, these formulations don't attract to the surface of the cells and CP permeated across the cell monolayer.

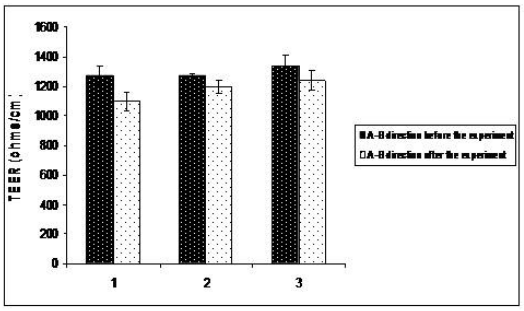
Transepithelial electrical resistance measurements

Following transport experiments, Caco-2 monolayers were examined with one way in this study. Transepithelial electrical resistances (TEER) of the cells were measured before and after the experiments. TEER can indicate irreversible

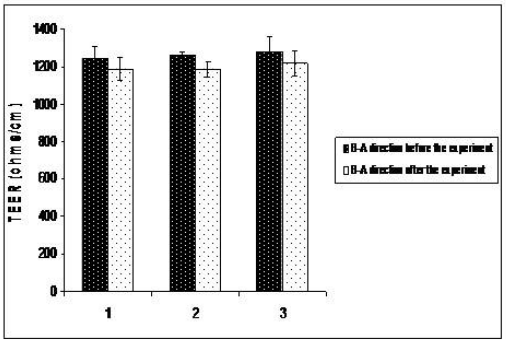
Table 3. The reduction % of TEER values of CP alone, CP-HP-B-CD complexes in the absence and presence of Na CMC with ±SD for A → B direction and B → A direction

Reduction % of TEER values		
Formulations	**A → B direction**	**B → A direction**
1	8.63 ±0.75 %	4.54 ±0.09 %
2	8.86 ±0.23 %	4.61 ±0.11 %
3	9.13 ±1.12%	4.89 ±0.23 %

*1- CP alone. 2- CP-HP-B-CD complex in the absence of Na CMC. 3- CP-HP-B-CD complex in the presence of Na CMC



(a) TEER values for A → B direction permeability studies for all formulations



(b) TEER values for B → A direction permeability studies for all formulations

Figure 8. TEER values measured before and after the experiments. (a) TEER values for A → B direction permeability studies for all formulations. (b) TEER values for B → A direction permeability studies for all formulations. 1- CP alone. 2- CP-HP-B-CD complex in the absence of Na CMC. 3- CP-HP-B-CD complex in the presence of Na CMC

membrane/cell damage. Therefore, minor toxicity cannot be detected with this parameter (36). In this study, a gastrointestinal cell culture model, the Caco-2 cell line, was used for the permeability studies of CP alone, CP-HP-β-CD complex in the absence and presence of Na CMC. The effect of these formulations was also investigated on the cell monolayer integrity and damage with TEER measurements. The TEER values of all experiments were found between 1207 ±81.76 and 1336 ±70.95 ohm/cm before the experiments for all formulations (Figure 8). After the experiments, the TEER values were found to be between 1100 ±39.16 and 1241 ±65.84 ohm/cm. A significant decrease in the TEER values was observed within from A → B direction for all formulations. The decrease of the TEER value was permeated concentrations of CP dependent in both directions, A → B and B → A. The effect of CP-HP-β-CD complex in the absence and presence of Na CMC on the TEER of Caco–2 cell monolayer is presented in Table 3. The results are presented as the percentage reducing of the TEER between the beginning and the end of the experiments. According to Table 3, all formulations are able to decrease the TEER value of Caco–2 cells to 8.63 ±0.75% and 9.13 ±1.12% from A → B direction. However, the percentage of TEER reduction from B → A direction was between 4.54 ±0.09% and 4.89 ±0.23% and less than from A → B direction. CP-HP-β-CD complex in the presence of Na CMC is more effective in reducing the TEER value than CP alone and CP-HP-β-CD complex in the absence of Na CMC. The TEER and permeability study results support each other. The permeability studies showed that the CP-HP-β-CD complex in the presence of Na CMC has a higher permeability compared to CP alone and CP-HP-β-CD complex in the absence of Na CMC. When the amount of permeated CP across Caco-2 cell monolayer is increased, the TEER values decreased. In addition, even if a decrease in TEER value was observed in the experiments, the cell monolayer was not damaged, because the TEER variation was not higher than 40% during to experiments (37). This demonstrated that the Caco–2 cells were still viable after the completion of all permeability experiments.

Although the detailed mechanism of CD action in enhancing the 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 the permeability of Caco-2 cell monolayers by displacing specific claudins from cholesterol rich domains associated with tight junctions (34).

CONCLUSIONS

Recently, various studies have been performed using CD complex formulation to improve solubility and permeability of drugs, which are of Class 4 in Biopharmaceutic Classification System (BCS). In this study, CP-HP-β-CD inclusion complexes were prepared in order to improve the poor solubility and permeability of CP that is a poor soluble and permeable drug. The solubility of CP was improved by an integrated complexation with 0.1% Na CMC and HP-β-CD. FTIR spectroscopy, DSC and SEM imaging revealed an interaction between CP and HP-β-CD in the complex. The new tablet formulation was designed by using CP-HP-β-CD inclusion complex and compared with CP bulk for permeability studies. The poor water solubility and permeability of CP were changed by an inclusion complexation with HP-β-CD. In addition, the permeability of CP was increased in the presence of complex formulation and Na CMC. The results suggest that:

– BCS Class 4 drug (such as CP) can be incorporated into the HP-β-CD complex formulation

– BCS Class 4 drug (such as CP) can be complexation with HP-β-CD in the presence of Na CMC

– This complexation (HP-β-CD complex and HP-β-CD complex in the presence of Na CMC) can be effective for the enhanced poor soluble and low permeable drug such as CP.

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