Acta Poloniae Pharmaceutica – Drug Research, Vol. 68 No. 3 pp. 423–433, 2011 ISSN 0001-6837
Polish Pharmaceutical Society

EVALUATION OF DRUG-EXCIPIENT INTERACTION IN THE FORMULATION OF CELECOXIB TABLETS

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Abstract: In the present study, the possible interactions between celecoxib and some excipients (colloidal silicon dioxide (Aerosil®), microcrystalline cellulose (Avicel® PH 102), lactose anhydrous, magnesium stearate, cross-povidone and talc) were evaluated by examining the pure drug or drug-excipient powder mixtures which were stored under different conditions (25 ± 2°C, 60% RH ± 5% RH or 40 ± 2°C, 75% RH ± 5% RH) and different period (30 or 60 days) using DSC, FT-IR and HPLC. In order to investigate the possibility of celecoxib-excipient interaction in aqueous medium, dispersions of the pure drug or drug in physical powder mixture (1:1 w/w) in water (1%, w/v) were also prepared and evaluated by FT-IR and HPLC at day 0 and day 7 (40 ± 2°C). The interaction between celecoxib and magnesium stearate or colloidal silicon dioxide were determined in the aqueous dispersions by FT-IR. Different tablet formulations with or without excipients tested were prepared, and assessed for drug dissolution and permeability.

Keywords: Celecoxib, drug-excipient interaction, permeability, Caco-2 cells

Interaction between drugs and excipients can alter stability and bioavailability of drugs, thereby, affecting its safety and/or efficacy (1). The successful formulation of a stable and effective solid dosage form depends on the careful choice of the excipients (2). The pharmaceutical development of solid dosage forms should imply a previous preformulation study of the drug and excipients (3). A number of experimental techniques (i.e., DSC, FT-IR spectroscopy, X-ray powder diffraction, Scanning Electron Microscopy, High Performance Liquid Chromatography (HPLC), etc.) have been used to investigate the interaction between drug and excipients (4, 5). These analyses can be applied to provide information on physicochemical properties of substances with respect to compatibility by predicting future problems of stability prior to the final solid dosage formulation (6).

Celecoxib (CXB), nonsteroidal anti-inflammatory drug (NSAID), is the first cyclooxygenase-2 (cox-2) inhibitor used in the treatment of osteoarthritis and rheumatoid arthritis in adult patients. In spite of its high gastrointestinal (GI) permeability, CXB shows incomplete and poor oral bioavailability. This could be attributed to low aqueous solubility of CXB, which causes inadequate dissolution in GI fluids and hence poor absorption, distribution, and target organ delivery (7).

A majority of the drug absorption in the gastrointestinal tract occurs in the small intestine (8). Human colon adenocarcinoma (Caco-2) cells are well characterized and currently most prevalent cell culture systems as a suitable *in vitro* model for the intestinal drug transport study (9). Caco-2 cells are also used to investigate the impact of excipients on drug absorption, involved mechanisms, absorption related drug-drug/excipient interaction, and associated cytotoxicities (8).

The aim of this study was to prepare CXB containing tablet formulations and determine the possible interactions between CXB and some excipients (colloidal silicon dioxide (Aerosil®), microcrystalline cellulose (Avicel® PH 102), lactose anhydrous; magnesium stearate, cross-povidone and talc) which are commonly used in solid dosage forms. Tablet formulations were also investigated to show the effect of drug-excipient interactions on dissolution and permeability profiles using human colon adenocarcinoma (Caco-2) cells.

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EXPERIMENTAL

Materials

Celecoxib was obtained from Fako Pharm. Co. (Turkey). Lactose anhydrous, magnesium stearate and talc were supplied from Merck (Germany). Colloidal silicon dioxide (Aerosil® 200) and microcrystalline cellulose (Avicel®PH 102) were purchased from Degussa (Germany) and Selectchemie AG (Switzerland), respectively. Cross-povidone was supplied from GAF Chem. (USA). Caco-2 cell lines were purchased from American Type Culture Collection (USA). L-glutamine, fetal bovine serum (FBS) and penicillin G sodium-streptomycin sulfate solution were provided by Biochrom (Germany). All other chemicals were obtained commercially as HPLC or analytical grade reagents.

Analytical methods

Assay of celecoxib

Determination of CXB was performed by HPLC according to the modified method of Abdel-Hamid et al. (10). The modular HPLC consisted of Hewlett Packard Co. Model 9012 constant flow pump, a Varian Co. Model 9100 autosampler and Hewlett Packard Co. chromatography workstation. Samples were analyzed on a Phenomenex Co. Nucleosil C_{18} column (5 mm particle size, 25 cm × 4.6 mm i.d.) and CXB was quantitated in a Varian Co. Model 9050 UV detector set at 254 nm. The mobile phase consisted of methanol : 1% acetic acid solution (80:20, v/v) and was run through the HPLC system at a rate of 1.5 mL/min at room temperature. The peak area used throughout this study and the chromatographic method were validated by linearity, sensitivity, precision, accuracy and specifity.

Differential scanning calorimetry (DSC)

DSC experiments were carried out with a DuPont Ins. 9900 Computer Thermal Analyzer (USA). Samples about 3 mg were weighed in pierced Al pans and scanned under static air over a temperature range of 25–205°C at a heating rate of 10°C/min, and the thermograms were reviewed for evidence of any interaction. Entalpy calculations were completed using TA-60 software (version 1.51).

Fourier transform-infrared spectroscopy (FT-IR)

Fourier Transform-Infrared Spectra were recorded on a Nicolet 520 (USA) apparatus using KBr discs in the range of 4000–400 cm^{-1}. The spectrum was a mean of ten consecutive scans on the same sample.

Formulation methods

Preparation of physical powder mixtures

In order to evaluate the CXB-excipient interaction in solid state mixture, physical powder mixtures of drug and some excipients were prepared. Microcrystalline cellulose (Avicel®PH 102) (MCC), colloidal silicon dioxide (Aerosil® 200) (CSD), lactose anhydrous (LA), magnesium stearate (MS), cross-povidone (CP) and talc (TA) were used as excipients. The drug : excipient (1:1, w/w, total amount: 500 g) were firstly homogeneously mixed with a pestle in a mortar, and blended in a laboratory powder mixer at 50 rpm (Erweka AR 400 Cube Blender, Berlin, Germany) for 10 min, then powder mixture was placed in a glass vial with rubber lid. The composition of the physical powder mixtures are shown in Table 1.

Preparation of dispersions of physical powder mixtures

Aqueous dispersions of physical powder mixtures of CXB and the excipients were prepared to examine the drug-excipient interaction in aqueous medium. The dispersions were prepared by addition of CXB (1%, w/v) or drug-excipient physical powder mixtures (1:1, w/w) (1%, w/v) to distilled water under continuous stirring until the powders were completely dispersed. The dispersions were stored at 40 ± 2°C for 7 days in an oven (Dedeoğlu, Turkey) and then filtered through Whatman filter paper No. 41 in order to collect dispersed particles. Afterwards, the particles were dried under ambient conditions to FT-IR analyses.

Preparation of tablets

In order to assess the CXB-excipient interaction in solid dosage form, tablet formulations containing excipients as the physical powder mixtures with different combination were produced. The compositions of the tablets prepared are presented in Table 1. Celecoxib, lactose anhydrous, microcrystalline cellulose and cross-povidone were mixed consecutively with mortar and pestle, and afterwards blended in a laboratory powder mixer at 50 rpm (Erweka AR 400 Cube Blender, Berlin, Germany) for 10 min. This powder mixture was blended with magnesium stearate and colloidal silicon dioxide (for formulation **F1**) or talc (for formulation **F2**) in a powder mixer (mixing time: 5 min). The resulting powder mixture was compressed into tablets at a compression force of 750 N and the speed of 10 mm/s using an eccentric compression machine (Erweka AR 400, Berlin, Germany) equipped with 12 mm circular flat-faced punches to

Table 1. Samples prepared for the formulations.

Type of sample	Code	Type of sample		Code
Sample as pure powder		*Sample as tablet formulation*		
CXB	CXB			
Colloidal silicon dioxide	CSD	*Formulation 1*	*w/w %*	
Microcrystalline cellulose	MCC	CXB	28.91	
Lactose (anhydrous)	LA	Lactose (anhydrous)	33.23	**F1**
Magnesium stearate	MS	Microcrystalline cellulose	28.91	
Cross-povidone	CP	Cross-povidone	7.22	
Talc	TA	Magnesium stearate	1.01	
		Colloidal silicon dioxide	0.72	
Sample as physical powder mixture (1:1, w/w)		*Formulation 2*	*w/w %*	
CXB + Colloidal silicon dioxide	P/CXB-CSD	CXB 28.91		
CXB + Microcrystalline cellulose	P/CXB-MCC	Lactose (anhydrous)	33.23	
CXB + Lactose (anhydrous)	P/CXB-LA	Microcrystalline cellulose	28.91	**F2**
CXB + Magnesium stearate	P/CXB-MS	Cross-povidone	7.22	
CXB + Cross-povidone	P/CXB-CP	Talc	1.73	
CXB + Talc	P/CXB-TA			
Sample as dispersion of the drug or physical powder mixture (1:1, w/w) in water (1%, w/v)				
CXB	D/CXB			
CXB + Colloidal silicon dioxide	D/CXB-CSD			
CXB + Microcrystalline cellulose	D/CXB-MCC			
CXB + Lactose (anhydrous)	D/CXB-LA			
CXB + Magnesium stearate	D/CXB-MS			
CXB + Cross-povidone	D/CXB-CP			
CXB + Talc	D/CXB-TA			

obtain 692 mg tablets. The resistance to crushing of tablets was 68 ± 1.5 N. The diametrical crushing strength of 20 tablets from each batch was measured using a bench-top hardness tester (Schleuniger-2E, Switzerland).

Conditions for storage study

One of the approaches to investigate the drug-excipient interaction is the conducting short-term stability studies using drug and excipients under stressed conditions (11). Thus, in the present study, the effect of temperature and humidity has been confirmed by examining the samples stored at different conditions (25 ± 2°C, 60% relative humidity (RH) ± 5% RH or 40 ± 2°C, 75% RH ± 5% RH) and at different periods (30 or 60 days). For this purpose, pure drug or the excipients, or physical powder mixtures or tablet formulations were stored for two months at 25 ± 2°C, 60% RH ± 5% RH or at 40 ± 2°C, 75% RH ± 5% RH in climate cabinet (Nüve EN 500, Turkey). Furthermore, the dispersions of drug or physical powder mixtures were stored in an oven as described above. The samples including powder mixtures were analyzed by DSC, FT-IR or HPLC assay (for CXB) in predetermined time points (0, 30, 60 and 90 days) while tablet formulations were examined for visual appearance and drug release properties at the same time points. The dispersions of physical powder mixtures were evaluated by FT-IR at day 0 and day 7.

***In vitro* dissolution study**

The drug dissolution study was performed in an USP XXIII paddle apparatus (Sotax AT 7 Smart Dissolution Testing Unit, Switzerland). The dissolution medium was tailored to maintain sink conditions throughout the dissolution testing and consisted of 900 mL sodium phosphate buffer with pH 7.4 and 2% (w/v) Tween-80 at 37 ± 0.5°C (12, 13). The rate of agitation of the paddle was 100 rpm. At predetermined time intervals (10, 20, 30, 60, 120, 180, 240 min) samples were withdrawn and filtered through a 0.45 μm filter and the concentration of CXB was determined by HPLC as described above.

Transport/absorption study with Caco-2 cells

Caco-2 cells were maintained in DMEM supplemented with 10% FBS, penicillin G sodium (50

units/mL) and streptomycin sulfate (50 µg/mL) at 37°C in a humidified incubator containing 5% CO_2. Cells were grown in 25 cm^2 culture flasks. Confluent cell monolayers were trypsinized with trypsin-EDTA solution (trypsin 0.005%, EDTA 0.002%, w/v) and the process was repeated through centrifugation, resuspension and incubation until introduced into transport wells.

In order to introduce Caco-2 cells into the inserts, 0.5 mL and 1.5 mL medium were transferred to the apical and basolateral sides of the wells, respectively. Cells suspensions from 24–26 passages were introduced into the insert at 6.00×10^4 cells/well (THIN CERTS 12 Well, Greiner bio-one, Germany, pore diameter 1 µm, surface 1.13 cm^2). The plate was then incubated at 5% CO_2, 37°C and humid incubator. Medium was changed on every 2nd day. Experiments were performed between 18–21 days after seeding, when the cell monolayer had reached confluence. Cell monolayer integrity was tested by measuring transepithelial resistance (TEER) with Millicell® ERS (Millipore, USA). When the resistance reached in the range of 400–600 ohm×cm^2, cell monolayer was used for transport studies.

As for Caco-2 cell transport studies, wells with TEER value of >400 ohm×cm^2 were selected. 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 cell monolayers were subsequently equilibrated for 30 min at 37°C. HBSS (0.5 mL) containing 10% DMSO (solution A) was used to prepare the samples to be placed the apical side of the monolayer. The solution of pure CXB (5 mg), dispersion of powder mixture of tablet formulation 1 containing 5 mg CXB in solution A or dispersion of powder mixture of tablet formulation 2 containing 5 mg CXB in solution A, was added to the apical side of the monolayer. One mL of 1% DMSO in 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 were analyzed by HPLC.

Apparent permeability co-efficient (cm/s) was calculated using Equation 1 (14):

$$P_{app} = dC/dt \cdot 1/(AC_0) \qquad \text{Eq.1}$$

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

Statistical analysis

Statistical analysis of data was performed with a *t*-test or one-way ANOVA using Statistica® software (Statsoft, Tulsa, OK, USA).

RESULTS AND DISCUSSION

Analytical methods

Assay of celecoxib

Calibration curve obtained for CXB was linear over the concentration range of 0.1–100 µg/mL. The limit of detection and quantitation were found to be 50 and 100 ng/mL, respectively. The intra- and inter-day precision relative standard deviation was 2.66% or less, and the accuracy was within 2.92% deviation of the nominal concentration. Furthermore, the HPLC method used was selective since no other peaks due to excipients in formulations on the retention time of CXB.

The results obtained from the HPLC analyses (n = 6, drug content and stability studies) of CXB revealed that pure drug or CXB in powder mixtures was stable during 30 and 60 days under both storage conditions since no significant changes in peak area of drug were noted ($p > 0.05$, see Table 2). There were no other peaks related to degradation products of drug or excipients. It can be deduced that the drug was stable in pure form or in the presence of excipients tested under these storage conditions.

Differential scanning calorimetry (DSC)

DSC scans of CXB and drug-excipient physical mixtures are shown in Figure 1. The thermal behavior of pure drug, respective excipient and combination of drug and excipient is compared in the DSC thermograms. The peak temperature and enthalpy values (ΔH (J/g)) for drug, excipient and drug-excipient mixture are summarized in Table 2.

The CXB presented its melting point at 163.75°C and enthalpy value of 93.28 J/g. Pure MCC, LA, MS (has two peaks) and CP exhibited shallow broad endothermic peaks at 58.93, 148.73, 94.64/118.15 and 65.14°C, respectively (Table 2), corresponding to their dehydration (unbound water). Furthermore, no peaks were observed for CSD and TA. When peak temperature and enthalpy values of pure excipients at day 0 were compared to those of the day 30 and day 60 for both of the storage conditions, no significant difference were observed for these values ($p > 0.05$).

The thermograms of P/CXB-CSD, P/CXB-MCC and P/CXB-TA showed an endothermic peak of drug at 163.55, 163.51 and 163.36°C indicating that there was no interaction (Figure 1). In the case of P/CXB-LA, P/CXB-MS and P/CXB-CP, endothermic peaks of LA, MS and CP showed a slight decrease in drug-excipient physical mixture, however no significant changes were observed in peak (at 163.88, 163.01 and 163.33°C, respectively)

Table 2. The assay results, peak temperature and enthalpy values (ΔH (J/g)) of CXB in pure form or in drug-excipient mixture during different storage conditions.

Code	Storage Conditions														
	initial			25 ± 2°C, 60% RH ± 5% RH						40 ± 2°C, 75% RH ± 5% RH					
				day 30			day 60			day 30			day 60		
	Peak Temp. (°C)	ΔH (J/g)	Assay (%)*	Peak Temp. (°C)	ΔH (J/g)	Assay (%)*	Peak Temp. (°C)	ΔH (J/g)	Assay (%)*	Peak Temp. (°C)	ΔH (J/g)	Assay (%)*	Peak Temp. (°C)	ΔH (J/g)	Assay (%)*
CXB	163.75	93.28	98.94 ± 0.35	163.34	105.70	105.23 ± 0.24	163.33	87.38	99.25 ± 0.19	163.83	150.8	100.09 ± 0.70	163.73	91.33	98.56 ± 0.22
CSD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MCC	58.93	-	-	58.97	-	-	58.99	-	-	58.85	-	-	58.91	-	-
LA	148.73	151.70	-	148.82	155.66	-	149.79	153.43	-	150.16	151.70	-	148.71	151.70	-
MS	118.15 /94.64	207.10	-	116.45 /95.73	208.17	-	118.98 /95.24	209.10	-	121.42 /98.66	213,57	-	118.02 /94.74	202.05	-
CP	65.14	-	-	66.48	-	-	65.89	-	-	68.57	-	-	65.06	-	-
TA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P/CXB-CSD	163.55	91.44	99.15 ± 0.14	163.31	92.50	98.18 ± 0.92	163.70	97.73	102.29 ± 0.26	163.46	95.01	98.07 ± 0.46	164.44	98.98	101.03 ± 0.21
P/CXB-MCC	163.51	93.85	99.18 ± 0.95	163.48	93.19	108.12 ± 0.16	163.44	97.16	102.01 ± 0.41	163.47	90.58	98.96 ± 0.13	163.46	93.16	100.15 ± 0.19
P/CXB-LA	163.88	94.04	98.40 ± 1.65	163.57	98.79	104.52 ± 0.07	163.36	90.70	102.23 ± 0.10	163.27	91.74	100.28 ± 0.33	164.29	90.43	102.66 ± 0.57
P/CXB-MS	163.05	93.51	103.72 ± 1.24	163.41	92.57	101.16 ± 0.14	163.56	96.10	100.8 ± 0.72	163.04	93.40	99.69 ± 0.18	163.62	93.89	100.40 ± 0.32
P/CXB-CP	163.33	96.16	103.19 ± 0.28	163.23	97.42	105.42 ± 0.09	163.28	92.10	101.79 ± 0.21	163.94	96.47	98.87 ± 0.27	163.42	97.45	103.62 ± 0.04
P/CXB-TA	163.36	93.67	97.88 ± 0.20	163.50	90.21	100.76 ± 0.24	164.38	92.88	98.35 ± 0.12	163.13	97.88	97.54 ± 0.20	163.59	91.02	99.46 ± 0.09

*(n = 6, X ± SD)

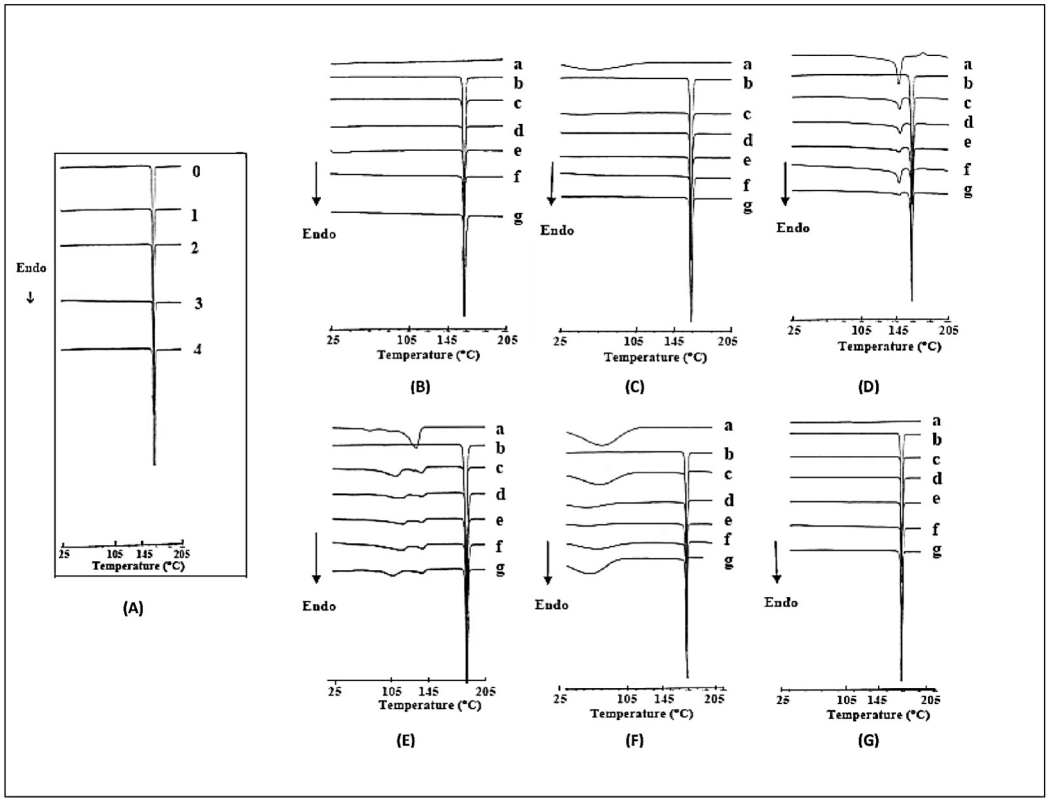

Figure 1. DSC scans of pure celecoxib, celecoxib-excipient physical mixtures at day 0, day 30 and day 60 for both storage conditions. **A**. Pure celecoxib: 0, at initial; 1, 2: at day 30 and day 60 (at 25 ± 2°C, 60% RH ± 5% RH); 3, 4: at day 30, day 60 (at 40 ± 2°C, 75% RH ± 5% RH). **B**, **C**, **D**, **E**, **F** and **G** for celecoxib-colloidal silicon dioxide, celecoxib-microcrystalline cellulose, celecoxib-lactose anhydrous, celecoxib-magnesium stearate, celecoxib-cross-povidone and celecoxib-talc physical mixtures, respectively. **a**, **b**, **c**: excipient, drug, drug-excipient mixtures at initial. **d**, **e**: excipient, drug, drug-excipient mixtures at day 30, day 60 (at 25 ± 2°C, 60% RH ± 5% RH). **f**, **g**: excipient, drug, drug-excipient mixtures at day 30, day 60 (at 40 ± 2°C, 75% RH ± 5% RH)

of the drug in these mixtures, which refers that there was no interaction. Also, this can be proved by the ΔH values of pure drug and drug-excipient mixtures since no significant changes were noted for ΔH values of these samples (see Table 2).

When melting point and enthalpy values of pure drug or CXB in powder mixtures at day 0 were compared to those of the day 30 and day 60 for both of the storage conditions, no significant difference were observed for these values ($p > 0.05$). Also, no significant changes in melting point and ΔH values of pure LA or MS were noted in the same storage conditions ($p > 0.05$) (see Table 2). Thus, it can be concluded that there was no interaction between drug and excipients under these conditions.

Fourier transform-infrared spectroscopy (FT-IR)

The selected FT-IR spectrums of CXB and samples of dispersions of physical powder mixtures are shown in Figure 2. The following characteristic streching bands were observed 3350–3220 (N–H), 1350-1180 (SO_2–N), 3050 (C–H, aromatic), 2950 (C–H, alkyl), 1600–1500 (C=C, aromatic) and 1350–1100 cm^{-1} (C-F) for pure CXB (Figure 2A), which were in accordance with the results of Bebawy et al. (15). When IR spectrum of pure CXB was compared to the spectrum of CXB in powder mixtures, no difference was observed between the spectra. Furthermore, neither missing in the bands nor appearance of new bands in the IR spectra of powder mixtures were noted at day 30 and day 60 for both storage conditions (data not shown). The HPLC assay results have confirmed that there were no change in the peak area of drug (see Table 2) and CXB was stable in the dry samples and no interaction were observed between drug and excipients under these storage conditions.

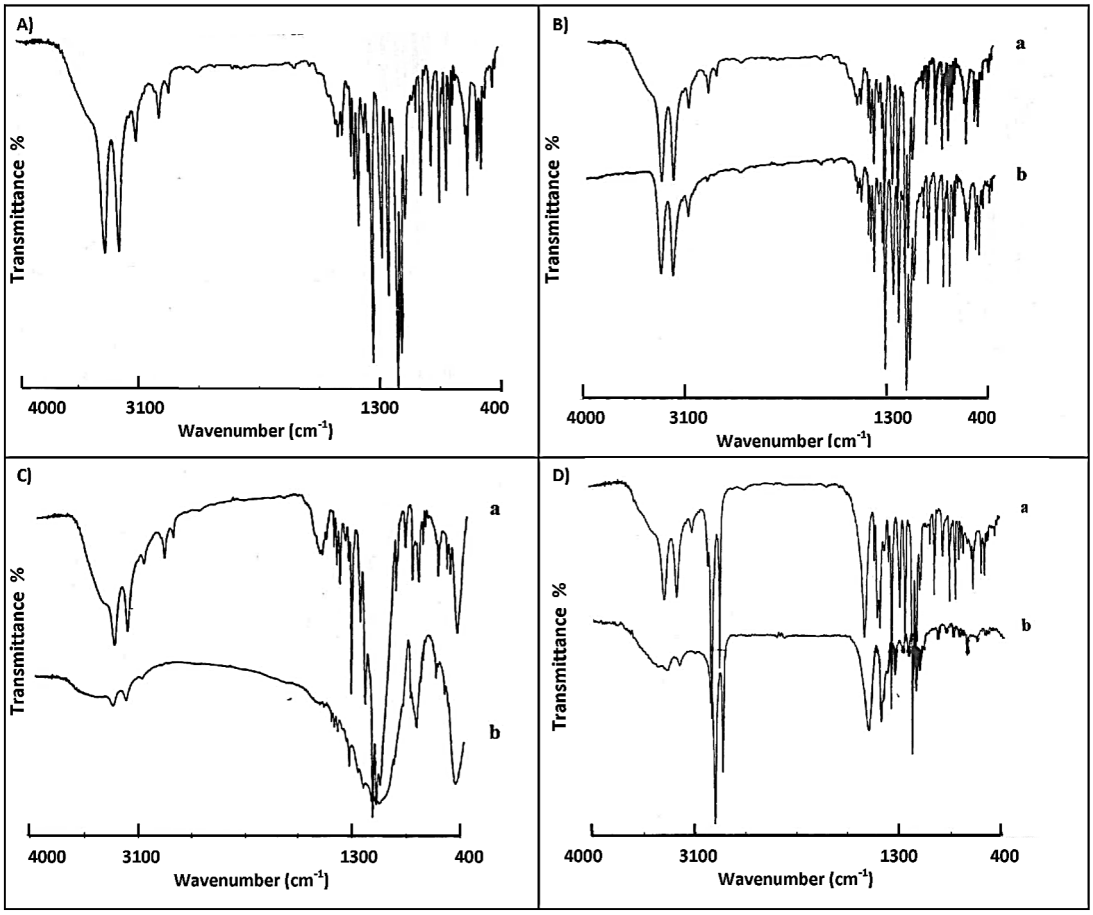

Figure 2. The selected FT-IR spectrums of pure celecoxib, dispersions of pure celecoxib and celecoxib-excipient physical mixtures. **A**. pure celecoxib; **B**. dispersion of pure celecoxib: **a**: at initial; **b**: at day 7 (40 ± 2°C). **C**. dispersion of physical mixtures of celecoxib-colloidal silicon dioxide: **a**: at initial; **b**: at day 7 (40 ± 2°C). **D**. dispersion of physical mixtures of celecoxib – magnesium stearate: **a**: at initial; **b**: at day 7 (40 ± 2°C)

In order to investigate the possibility of CXB-excipient interaction in aqueous medium, dispersions of the pure drug or drug in physical powder mixture (1:1, w/w) in water (1%, w/v) were prepared and evaluated by FT-IR and HPLC at day 0 and day 7 (40 ± 2°C). No differences were observed in FT-IR spectrums of all the dispersions at day 0. However, when IR spectrum of pure CXB dispersion (D/CXB) (Figure 2B) was compared to the spectra of CXB in powder mixture dispersions (D/CXB-MCC, D/CXB-LA, D/CXB-CP, D/CXB-CSD, D/CXB-MS and D/CXB-TA), no differences were observed between the spectra except the samples containing colloidal silicon dioxide (D/CXB-CSD) or magnesium stearate (D/CXB-MS) (Figure 2C-b and 2D-b). For both of these samples, the weaker signals were noted in 3350–3220 (N–H) and 1350–1180 cm^{-1} (SO_2–N) bands in the IR spectrum compared to those of D/CXB (pure drug dispersion). Bebawy et al. (15) evaluated the degradation products of CXB under stressed conditions and reported that when degradation products of CXB occured, 3350–3220 (N–H) and 1350–1180 (SO_2–N) cm^{-1} bands were missing in the IR spectrum of the drug. In our study, however, weaker signals were noted in these bands in D/CXB-CSD or D/CXB-MS samples and there was no change in the chromatographic behavior of drug in its pure form and drug-excipient mixture. Also, quantitative results after 7 days of storage at 40 ± 2°C ruled out any drug degradation (assay results were 103.6 ± 2.2% and 99.8 ± 1.4% for D/CXB-CSD and D/CXB-MS, respectively). The weaker signals in the IR spectrum could be attributed to the chemical interaction between drug and CSD or MS. The decrease in strenght of the -NH and SO_2–N bands of CBX suggested that the exis-

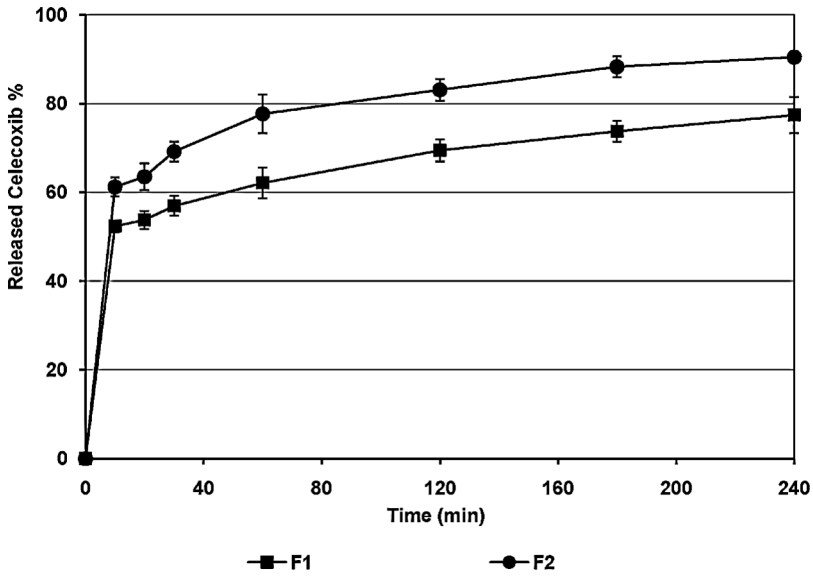

Figure 3. The *in vitro* dissolution profiles of celecoxib from formulation **F1** and **F2** (n = 6, X ± SD)

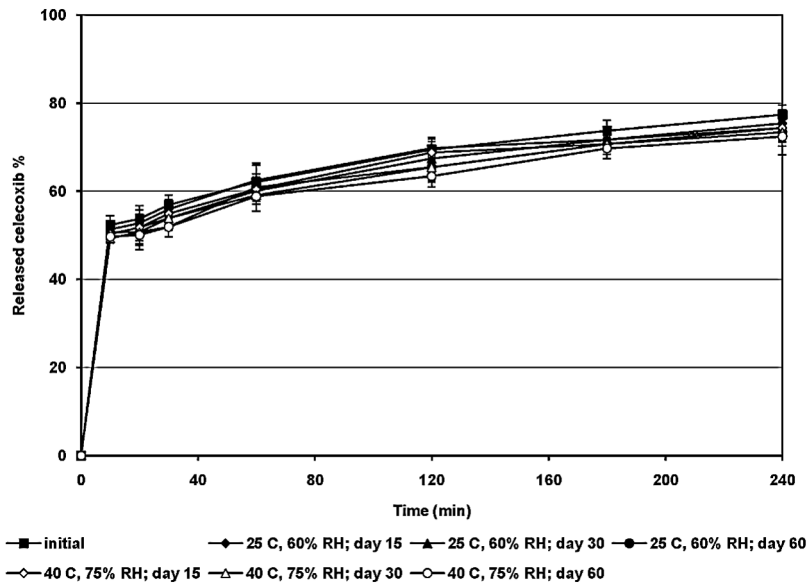

Figure 4. The *in vitro* dissolution profiles of celecoxib from formulation **F1** at day 0, day 15, day 30 and day 60 for both storage conditions (n = 6, X ± SD)

tence of formation of hydrogen bonding between the sulfonamide group of CBX and the carboxyl groups of MS (magnesium stearate) or silanol groups of CSD (colloidal silicon dioxide). Further studies will be performed to elucidate this interaction in detail. Besides, these results are in agreement with the work of Sinha et al. (16) who observed the formation of hydrogen bonding between the sulfonamide group of CBX and the hydroxyl groups of β-cyclodextrin in solution by using FT-IR.

Formulation development and drug dissolution studies

Despite the importance of drug-excipient interaction, there is no universally accepted protocol for this purpose. In this study, 1:1 ratio was chosen as a standard approach in order to maximize the likelihood of observing any interaction (1, 17). Also, it was demonstrated that the type and level of excipient influenced the characterization of the tablets (18, 19). For this reason, the complete formulations, in

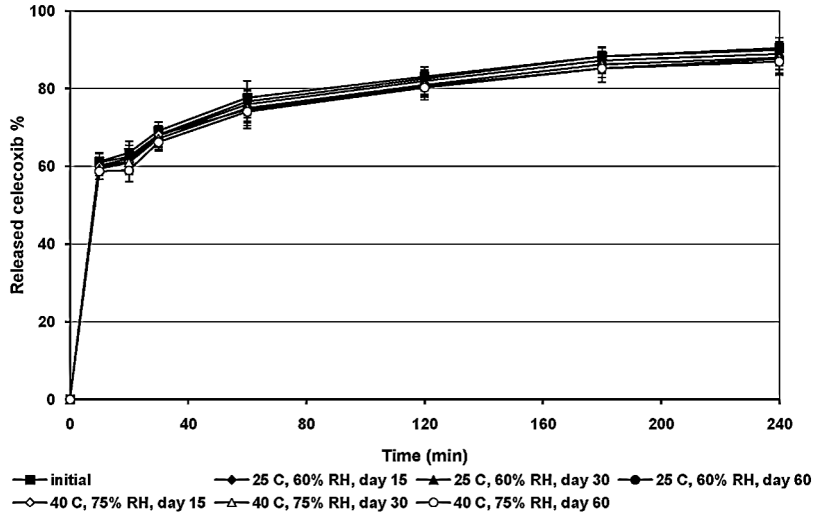

Figure 5. The *in vitro* dissolution profiles of celecoxib from formulation **F2** at day 0, day 15, day 30 and day 60 for both storage conditions (n = 6, X ± SD)

Table 3. Permeability (apical to basolateral) of CXB*

Formulation	$P_{Caco\text{-}2} \times 10^{-6}$ cm/s
	A to B
Pure CXB	1.54 ± 1.09
F1	0.82 ± 0.45
F2	82.7 ± 27.6

* (n = 3, X ± SD)

the presence of the excipients in usual application concentration, were investigated, in order to have outcome of the behavior of the tablets under study conditions.

The excipients which were evaluated by DSC, FT-IR and HPLC studies were used for the preparation of tablet formulations (see Table 1). The percentages of CXB, LA, MCC and CP were the same for both of the formulations (**F1** and **F2**). As FT-IR studies (see above) showed that there was an interaction between CXB and MS or CSD, both of the excipients were used in formulation **F1** whether this interaction could be effective on the dissolution of drug during storage time period (day 0, 30 and 60) at different conditions (25 ± 2°C, 60% relative humidity (RH) ± 5% RH or at 40 ± 2°C, 75% RH ± 5% RH). For comparison, since no interaction was detected between TA and CXB, TA was used for formulation **F2** with the total percentages of MS and CSD.

The *in vitro* dissolution profiles of CXB from formulation **F1** and **F2** are presented in Figure 3. The slower CXB dissolution was observed in formulation **F1** compared to **F2**. Ninety percent of the drug was dissolved in 4 h from formulation **F2**, while 77% of CXB was dissolved from **F1** in the same period. The slower dissolution of CXB from **F1** can be explained by the interaction between drug and MS or CSD which was determined in FT-IR study. Also, it could be due to hydrophobic nature of MS and CSD combination compared to that of talc. MS is well known as a highly hydrophobic lubricant (20, 21), so that the disintegration time of the tablets containing MS is longer than that of others. Furthermore, talc has hydrophobic and hydrophilic properties because it can be dispersed into aqueous media and oil (22). Kuno et al. (20) have evaluated the effect of lubricants on the characteristics of orally disintegrating tablets and reported that disintegration time of tablets including talc is faster than that of MS due to higher water uptake of formulations containing talc compared to the others. Little information is, however, available about the CXB-excipient interaction in different dosage (23–26).

The *in vitro* dissolution profiles of CXB from formulation **F1** and **F2** during 60 days of storage for both conditions are presented in Figures 4 and 5. No significant changes were observed in dissolution profiles of formulation **F1** and **F2** during storage period. The results suggest that temperature and humidity did not have an effect on the dissolution properties of the tablets.

Transport/absorption study with Caco-2 cells

In the present study, Caco-2 cells were used in order to investigate the influence of selected excipients on CXB permeability. Since the results

obtained from the FT-IR studies indicated that there was an interaction between CXB and MS or CSD, two tablet formulations were prepared containing either MS-CSD combination or TA (which was not interacting with the drug) as lubricant in order to evaluate the excipients effects on tablet characteristics such as drug dissolution and permeation. Therefore, the solution of pure drug or dispersions of powder mixtures, which were used for the preparation of formulation **F1** or **F2**, were used in the transport studies.

As it is seen in Table 3, the permeability (apical to basolateral) of CXB in pure drug solution was 1,54 (± 1.09) × 10^{-6} cm/s while that was 0,82 (± 0,45) and 82,7 (± 27,6) × 10^{-6} cm/s for **F1** and **F2** formulation, respectively. The permeability of pure drug through Caco-2 cells was in accordance with the study of Venture et al. (27). Furthermore, while permeability of drug was showing approximately 53 fold increase in formulation **F2**, that was the approximately half of the pure drug permeability in formulation **F1**.

Working with sensitive cell culture models such as Caco-2 cell monolayers puts limitations on the use of the aqueous media being in contact with them. Viability and integrity of a Caco-2 monolayer depends on isosmotic pressure in combination with appropriate pH values, and thus choice of media which is directly led over the Caco-2 monolayer. Furthermore, such permeability assays are normally carried out with pure and completely dissolved compounds, and thus lack the possibility to monitor influences of the dosage form and excipients on permeability (28). In the present study, while significant differences were observed between permeability of pure drug and the drug in formulations, no significant changes were obtained in osmolality and pH values of pure drug solution and dispersion of the formulations (data not shown). Therefore, lower permeability of CXB in formulation **F1** could be attributed to interaction between drug and MS or CSD and the hydrophobic nature of MS and CSD combination compared to that of talc as mentioned above. Hydrophobic interaction between CXB (hydrophobic drug) and MS-CSD combination creates a hydrophobic stationary environment and this will cause retention of drug molecules in the apical side decreasing the permeability for the **F1** formulation. Furthermore, when the tablets contact with water, more hydrophilic layer can be formed by talc in formulation **F2**, which accelerates the dissolution of the drug, and rapid dissolution of the drug could have a positive effect on the permeability. Similarly, Venture et al. (27) prepared CXB and 2,6-di-O-methyl-β-cyclodextrin complexes in order to increase the drug solubility and reported that the complexation produced a significant increase of water solubility of CXB, which is able to increase the permeation of CXB across a CaCo-2 cell monolayer, with respect to the free drug. For present study, further work is needed to interpret the differences in permeability of pure drug and drug in formulations in detail.

CONCLUSION

Traditionally, excipients have been regarded as inert. However, there are many instances in which excipients have been shown to have a significant effect on the biological availability of the drug. Positive (*via* complexation of drug with cyclodextrins or solid dispersion technology) or negative effect on the drug bioavailability can be seen with a drug-excipient interaction.

In this study, excipients, which were commonly used in solid drug formulations, were evaluated for interaction possibility with CXB. The results of DSC along with FT-IR or HPLC studies showed that there were no interactions between drug and selected excipients during 30 and 60 days under both storage conditions and also, pure drug or CXB in powder mixtures was stable during the tests under these conditions. On the other hand, no drug degradation but the interaction between CXB and MS or CSD was observed in aqueous medium with FT-IR study, which indicates that drug-excipient interaction studies should be done in aqueous medium in addition to solid mixture.

Two tablet formulations were prepared including either MS-CSD combination or TA (which was no interact with the drug) as lubricant in order to evaluate the effects of excipients on some of the tablet characteristics. The results obtained from the drug dissolution studies showed that the interaction between CXB and MS or CSD and hydrophobicity of excipients had an effect of the dissolution of drug. Faster drug dissolution for formulation **F2** was noted compared to that for formulation **F1** due to drug-excipient interaction and the difference in hydrophobicity of the formulations. Furthermore, the dissolution profiles of formulation **F1** and **F2** remained unaffected after storage period. The permeability studies showed that the significant differences were observed between permeability of pure drug and the drug in formulations, which could be attributed the interaction between drug and MS or CSD and the hydrophobic nature of MS and CSD combination compared to that of talc.

Also further work is necessary to explain the differences in permeability of pure drug and drug in formulations in detail.

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Received: 15. 11. 2010