science, engineering and health studies

Research Article

Science, Engineering and Health Studies
https://lio1.tci-thaijo.org/index.php/sehs
ISSN (Online): 2630-0087

Formulation and characterization of piroxicam/cyclodextrin taste masked oral lyophilisates

Phennapha Saokham[1], Siripat Chaichit[2], and Kanokporn Burapapadh[3]*

[1] Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand
[2] Laboratory for Molecular Design and Simulation (LMDS), Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand
[3] Department of Manufacturing Pharmacy, College of Pharmacy, Rangsit University, Pathum Thani 12000, Thailand

***Corresponding author**:
Kanokporn Burapapadh
kanokporn.b@rsu.ac.th

***Received**: 11 April 2023*
***Revised**: 2 June 2023*
***Accepted**: 7 June 2023*
***Published**: 20 November 2023*

***Citation**:
Saokham, P., Chaichit, S., and Burapapadh, K. (2023). Formulation and characterization of piroxicam/cyclodextrin taste masked oral lyophilisates. Science, Engineering and Health Studies, 17, 23050008.

ABSTRACT

Oral lyophilisates are one of the orodispersible tablets produced by lyophilization technique. Due to their porous structure, they instantly disintegrate when contacting saliva. Generally, they are suitable for drugs with a short onset of action, such as nonsteroidal anti-inflammatory drugs (NSAIDs). Therefore, formulations of piroxicam lyophilisates and their properties were investigated. Because piroxicam is classified as a low solubility but high permeability drug, a solubility enhancing agent should be included in the formulation. The influence of β-cyclodextrin (βCD) and hydroxypropyl-β-cyclodextrin (HPβCD) on disintegration time and morphology of piroxicam lyophilisates was studied. Furthermore, the satisfactory taste-masking efficiency of HPβCD in piroxicam lyophilisates was studied by sensory test. The effect of matrix polymer mixtures on disintegration time was previously evaluated. The appropriate ratio of polymer mixture, i.e., gelatin and hydroxypropyl methylcellulose (HPMC), provided lyophilisates with the fastest disintegration time (<30 s) was designated for further studies. The presence of both CDs decreased disintegration time and modified the surface and matrix of lyophilisates. With the theoretical study based on molecular mechanics methods and the investigation through the chemical shift of protons using nuclear magnetic resonance (NMR), piroxicam formed inclusion complexes with both βCD and HPβCD. Dissolution profiles of piroxicam/HPβCD lyophilisates revealed improved piroxicam solubility by inclusion complex formation. Finally, the piroxicam/HPβCD lyophilisates demonstrate the bitterness suppression in healthy volunteers.

Keywords: cyclodextrin; orodispersible tablets; piroxicam; taste masking; molecular docking

1. INTRODUCTION

Orodispersible dosage forms are a novel technology where the dosage form disintegrates or dissolves rapidly within the mouth without the need for water. It disintegrates after contacting saliva and completely turns into a solution or suspension within 30 to 50 s (Ciper and Bodmeier, 2006; Mizumoto et al., 2005). The active pharmaceutical ingredients (APIs) are then swallowed and absorbed through the gastrointestinal epithelium. Tablets are the most favorable among these dosage forms. They are compact and provide a very accurate dose administration even of low dose drugs. Considering the patient perspective, this dosage form is self-administered and convenient for handling, contributing to better patient compliance, especially among the elderly and children. Various techniques can be used to manufacture orodispersible tablets, such as direct

compression, molding, granulation, etc. However, the most famous and successful method is lyophilization or freeze drying (Parkash et al., 2011). This process provides a light, porous structures called lyophilisates (Chandrasekhar et al., 2009; Iurian et al., 2017). The fabrication of oral lyophilisates is based on the sublimation of water from frozen aqueous formulation. The matrix of lyophilisate formulation regularly consists of a water soluble polymer, e.g., gelatin, dextran, alginate, maltodextrin (Seager, 1998) or amino acids (AlHusban et al., 2010) and matrix supporting agents, e.g., sucrose or mannitol. The use of saccharides and polyols should be considered due to the recommended daily allowance and the medical concerns of individual patients. Therefore, the feasibility of the polymer mixture compensating for an adequate disintegration time of oral lyophilisates was first studied.

Cyclodextrins (CDs) are cyclic oligosaccharides connecting six, seven, or eight units of D-glucopyranose with α-1,4 linkage. It possesses a truncated cone shape with a hydrophilic outer surface and a hydrophobic cavity forming noncovalent bonding with a lipophilic moiety of APIs. The intermolecular interactions with secondary or primary rim, particularly van der Waals, and hydrophobic interactions, within the cavity with the nonpolar groups of guests induce the formation of water-soluble host-guest inclusion complexes. The APIs can form host-guest inclusion complexes with possibly different stoichiometries, molecular conformation, and equilibrium constants (Raffaini and Ganazzoli, 2020).

The phase solubility study is a simple quantitative method used to determine the solubility of API in the presence of aqueous CD solutions. When the solubility of API increases with increasing CD concentration, the formation of API/CD inclusion complexes occurs. According to Higuchi and Connors classification (Higuchi and Connors, 1965), the plot of solubility against concentration of CD (so called phase solubility profile) is usually classified as A_L- or B_S-type depending on the structure of the CD and API. The stoichiometry of the API/CD inclusion complex and its stability constant are also obtained from the phase solubility profile.

Nuclear magnetic resonance (NMR) spectroscopy, especially proton NMR (^{1}H NMR), is one of the most effective tools to study complex formations (Fronza et al., 1992; Redenti et al., 1999). In the liquid state, the physicochemical properties of API and CD change on forming the inclusion complex through intermolecular interactions with a few angstroms distance. NMR parameters such as chemical shift are sensitive to short-range intermolecular interactions. Therefore, the CD complex phenomena in liquid can be observed by the changing proton chemical shift. To obtain the complex information, the full assignments of all resonances of API and CD appearing in the spectra are required. Complexation-induced changes in chemical shifts of both API and CD are then studied. The inclusion of API induces changes in proton chemical shift located inside the CD cavity, i.e., H-3 and H-5 (Figure 1(a)), due to anisotropic shielding. They also reflect the location of included the API corresponding to protons (Inoue, 1993).

Molecular docking is one of the favorable theoretical computational chemistry techniques used to predict the geometries and interaction energy of CD complexes. It can provide the probabilities of different preferred conformations with individual interaction energy. The selection of reliable conformation can then apply. Combined experimental and theoretical studies have been recognized as a useful technique to provide information on the CD inclusion complex (Alcaro et al., 2002; de Sousa et al., 2008; Han et al., 2020; Madi et al., 2009; Snor et al., 2009).

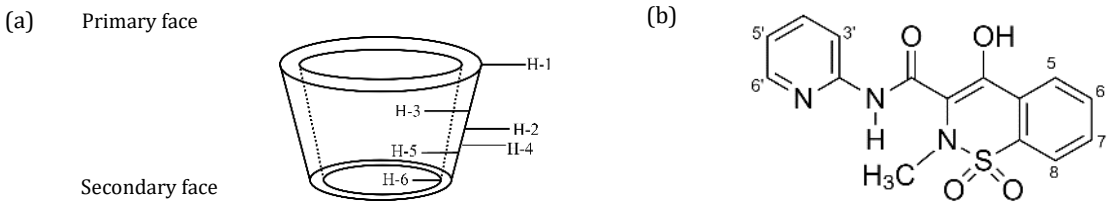

Figure 1. A schematic representation of (a) CD and (b) chemical structure of piroxicam

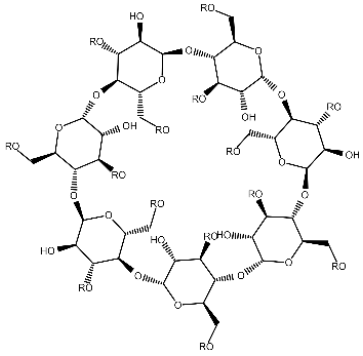

Figure 2. Structure of βCD (R = hydrogen (-H)) and HPβCD (R = hydroxypropyl (-$CH_2CH(OH)CH_3$)), which composed of seven glucopyranose units

Piroxicam is an oxicam nonsteroidal anti-inflammatory drug (Figure 1(b)), which is widely used in musculoskeletal and joint disorders, and is practically insoluble in water (Mostafa et al., 2020). It belongs to Class II of the Biopharmaceutical Classification System (Mirza et al., 2010), i.e., having high permeability but low aqueous solubility. Thus, the bioavailability of piroxicam is limited by its solubility and dissolution rates. To increase those two properties, drug/cyclodextrin inclusion complexes are of interest. Complexation of piroxicam with βCD (Figure 2) has been used for more than 30 years. After administering, these complexes potentially provide rapid onset of action and minimize drug gastric effects resulting in improved gastrointestinal tolerability (Scarpignato, 2013). Numerous studies about the properties and applications of piroxicam/βCD inclusion complexes have been reported (Braibanti et al., 1998; Fronza et al., 1992; Mirza et al., 2010; Mostafa et al., 2020; Scarpignato, 2013). One of the favorable βCD derivatives is hydroxypropyl-β-cyclodextrin (HPβCD) which involves βCD substitutes with 2-hydroxypropyl at the hydroxy groups of D-glucopyranose subunits (Figure 2). Like βCD, piroxicam also forms inclusion complexes with HPβCD (Xiliang et al., 2003). Even though their inclusion complexation was studied in various dosage forms, e.g., topical gel (Doliwa et al., 2001), mucoadhesive tablet (Jug and Bećirević-Laćan, 2004) and sublingual nanofiber (Topuz, 2022), and techniques, e.g., supercritical fluid (Banchero and Manna, 2011), solid dispersion (Bouchal et al., 2015), electrospinning (Topuz, 2022) and fluid bed coating (Zhang et al., 2009), the studies of piroxicam in orodispersible tablets prepared by lyophilization (or lyophilisates) are limited. Moreover, related literature proposed that the presence of auxiliary or ternary agents such as polyvinyl polyvinylpyrrolidone (PVP), L-lysine (Banchero and Manna, 2011) and polyethylene glycol (PEG) 6000 (Bouchal et al., 2015) affect the behavior of piroxicam/HPβCD in the solid state. Although in a solid state geometry can explain many characteristics of the liquid state, the geometry of the complex in liquid state is not necessary, the same as that in the solid state (Inoue, 1993). Also, the information of the complex in the solid state demonstrated the complex formation in lyophilisates, but not in the medium when those lyophilsates disintegrate in the saliva, then dissolve in the gastrointestinal tract, i.e., in the liquid state. Hence, in this study, lyophilisates containing piroxicam were prepared, and cyclodextrin, i.e., βCD and HPβCD, were used to facilitate solubility and dissolution of the drug. The inclusion complex information in the liquid state was investigated using phase solubility studies, ^{1}H NMR, and molecular docking. The ability to mask the bitter taste of cyclodextrin was also studied using a sensory test.

2. MATERIALS AND METHODS

2.1 Materials

Hydroxypropyl methylcellulose (HPMC, Pharmacoat® 606, Shin-Etsu) was kindly donated by True Solutrade, Co., Ltd., Bangkok, Thailand, Gelatin 250 Bloom and polyethylene glycol 400 were purchased from P.C. Drug Center Co., Ltd., Thailand and Acros Organics, USA, respectively. β-cyclodextrin (βCD) and HPβCD (degree of substitution 0.6-0.9) were purchased from Tokyo Chemical Industry Co., Ltd., Japan. Piroxicam was obtained from Apex Healthcare Limited, India. Methanol (AR grade) and concentrated hydrochloric acid (36.5-38.0%, A.C.S reagent), used as analysis solvents, were purchased from RCI Labscan Limited, Thailand, and J.T.Baker™, USA, respectively. Deionized (DI) water was used as a solvent for preparation and dissolution studies. Other chemicals, such as sodium chloride and potassium phosphate monobasic, were analytical grade.

2.2 Phase solubility studies of piroxicam in aqueous βCD and HPβCD solutions

For drug/CD inclusion complexes, their phase-solubility profiles describe how the apparent solubility of a drug changes in the presence of CD through complex formation (Higuchi and Connors, 1965). Saturated solutions of piroxicam in various concentrations of βCD or HPβCD were prepared in triplicate. Briefly, the excess amount of piroxicam was added to aqueous solutions containing 0 to 13 mM βCD or 0 to 20 mM HPβCD (pH 6.5-6.8). Those suspensions were mixed vigorously using a Vortex mixer (Vortex-Genie 2, Scientific Industries, Inc., USA) in 10 mL glass test tubes and allowed to equilibrate at 25±5°C for 7 days under horizontal agitation at 200 rpm (WiseCube® Shaking Incubator WIS-20R, Daihan Scientific Co., Ltd., South Korea). After achieving equilibrium, the suspensions were filtered through a 0.45 µm syringe membrane filter (VertiPure® nylon syringe filter, Ligand Scientific, Thailand). The concentration of piroxicam in the filtrate was determined using a UV-visible spectrophotometer (Evolution 201 UV-Visible spectrophotometer, Thermo Scientific, USA) at a wavelength of 254 nm. Each sample was employed in triplicate. The solubility of piroxicam was then computed with a calibration curve ranging from 10 to 40 µm/mL of piroxicam in a 0.01N methanolic hydrochloric acid solution ($R^2 > 0.999$). The limit of detection (LOD) and limit of quantification (LOQ) were 1.35 and 4.08 µm/mL, respectively. The aqueous CD solutions were used as blanks. Phase-solubility profiles were plotted according to the method of Higuchi and Connors (Higuchi and Connors, 1965). When slope is less than unity, it could be assumed that the drug/CD complex is a 1:1 drug/CD complex (Loftsson and Brewster, 2010), where the piroxicam molecule (P) forms a complex with one CD molecule:

$$P + CD \overset{K_{1:1}}{\rightleftarrows} P/CD \quad (1)$$

The apparent stability constant ($K_{1:1}$) and the complexation efficiency (CE) were determined using the slope of the linear region of phase-solubility profiles (Loftsson et al., 2005):

$$K_{1:1} = \frac{\text{slope}}{S_0(1 - \text{slope})} \quad (2)$$

$$CE = \frac{\text{slope}}{1 - \text{slope}} = \frac{[\text{P/CD complex}]}{[\text{CD}]} = K_{1:1}S_0 \quad (3)$$

where S_0 is the determined intrinsic solubility of the piroxicam, slope is the slope of the linear region, [P/CD complex] is the concentration of dissolved piroxicam/CD complex, and [CD] is the concentration of dissolved free cyclodextrin, i.e., βCD or HPβCD. The CE could be used to

calculate the piroxicam:CD (P:CD) ratio, which could be correlated to the expected increase in formulation bulk (Loftsson and Brewster, 2010; Loftsson et al., 2007):

$$P : CD = 1 : \left(1 + \frac{1}{CE}\right) \quad (4)$$

2.3 Molecular docking studies

The 3D structure data of βCD (PDB ID: 1Z0N) (Polekhina et al., 2005) was downloaded from the Protein Data Bank. The 3D structure of HPβCD was constructed from the structure of βCD then geometry optimized using Gaussian Software at the PM6 level. All structures were input in BIOVIA Discovery Studio Visualizer Software (Dassault Systèmes, 2021) to create a PDB file format. Molecular docking simulations were carried out using AutoDock 4.2 Software (Scripps Research Institute, La Jolla, CA, USA)(Morris et al., 2009). All hydrogen and Gasteiger charges were added to prepare the macromolecule (βCD or HPβCD) and ligand (piroxicam) in PDBQT file format. Based on the dimensions of CD, grid maps were 60 × 60 × 60 points with a grid point spacing of 0.375 Å to allow the piroxicam to rotate freely. The Lamarckian genetic algorithm (LGA) with 100 separated docking runs and a maximum of 25,000,000 energy evaluations were performed for docking calculations. All the other parameters were the default values set by AutoDock. The conformations with the minimum binding energy in the highest populated clusters were selected as the best docking poses and the most stable binding model. PyMOL 2.5 (Schrödinger, 2021) was finally used to visualize the 3D interaction between piroxicam and CDs.

2.4 Preparation of lyophilisates

Before piroxicam loading, formulations of blank lyophilisates were investigated. Aqueous solutions of gelatin or HPMC at a concentration of 1% w/w were individually prepared. The required amount of HPMC was dispersed in DI water under magnetic stirring. Gelatin was previously dispersed in 60±5°C DI water until completely dissolved, then cooled down to room temperature. The optimal ratio of the polymer mixture was then studied. Various ratios of gelatin:HPMC, i.e., 1:1, 1:4, 2:3, 3:2, and 4:1 were prepared by adding aqueous HPMC solution to the gelatin solution. The total concentration of polymer mixture was fixed at 2% w/w. After the optimum ratio of the polymer mixture was selected, the 1% w/w of piroxicam was dispersed. An assigned quantity of other excipients such as PEG400 (0.1-0.3% w/w), citric acid (0.75% w/w), erythritol (0.5-1% w/w) and stevioside (0.5% w/w) was thoroughly mixed at least 2 h. Furthermore, a 1 mL aqueous polymer solution or piroxicam polymer suspension (equivalent to piroxicam 10 mg) was added to the polystyrene molds (diameter 15.5 mm) (SPL Life Sciences Co., Ltd., South Korea) as reported in related studies (Boateng et al., 2012; Farias and Boateng, 2018; Kianfar et al., 2014; Mura et al., 2015). The lyophilisates were prepared by freezing the molds at −75 °C (Scancool Snowbird Ultra-Freezer, Labogene, Denmark) for 24 h, then transferred to an Alpha 2–4 LCSplus lyophilizer (Christ, Germany) for drying over a 48-h period. The lyophilisates were then discarded from the molds and stored in a desiccator. The promising formulation was selected to investigate the effect of piroxicam/βCD and piroxicam/HPβCD inclusion complexes on the disintegration times, dissolution profiles and release mechanisms of piroxicam.

2.5 Morphology and physicochemical properties studies of lyophilisates

2.5.1 Scanning electron microscopy (SEM)

The lyophilisates were fixed on stubs with double-side, adhesive tape to observe their surface and cross-sectional view morphology. The fixed stubs were coated with thin gold layer before investigating using an SEM (Mira3, Tescan, Czech Republic) at magnifications of 10000× and 50000×.

2.5.2 In vitro disintegration time

A lyophilisate was placed in 2-mL of pH 6.8±0.2 artificial saliva (consisting of 8 g/L sodium chloride, 0.19 g/L potassium phosphate monobasic and 2.83 g/L sodium phosphate dibasic, then adjusted to pH 6.8 with 1N hydrochloric acid)(Marques et al., 2011) drop on the 100 x 20 mm, glass petri dish. Disintegration time was defined when the lyophilisate was completely dissolved in media or disintegrated into small pieces. An average and a standard deviation of three measurements were calculated.

2.5.3 Proton nuclear magnetic resonance (^{1}H-NMR) analysis

To investigate complex formation in the liquid state, the ^{1}H-NMR spectra were recorded at 500 MHz (AVANCE NEO, Bruker, Switzerland). To investigate the formation of piroxicam/CD complex in the liquid-state, approximately 10 mg of free compounds, i.e., piroxicam, βCD and HPβCD, and solid complexes at a ratio of 1:1, i.e., piroxicam/βCD and piroxicam/HPβCD, were dispersed in DI water and stirred for 2 h using a magnetic stirrer. The suspensions or solutions were dried in a lyophilizer. All dried compounds and solid complexes were dissolved in dimethyl sulfoxide-d6 (DMSO-d6) relating to the signal at 2.50 ppm. The ^{1}H chemical shifts of piroxicam, βCD, and HPβCD were first assigned, then determined for changes in those chemical shifts (Δδ) according to the following equation:

$$\Delta\delta = \delta_{complex} - \delta_{reference} \quad (5)$$

Furthermore, the formation of piroxicam/CD in composite excipient solution was studied. According to the formulation development, six different lyophilisates were prepared. Lyophilisates containing piroxicam/βCD or piroxicam/HPβCD were applied as a complex, while lyophilisates with piroxicam, βCD, or HPβCD served as a reference. Lyophilisates without piroxicam, βCD, and HPβCD were assigned as blank to determine any signals from excipient.

2.5.4 *In vitro* dissolution studies of piroxicam

The dissolution studies of piroxicam were conducted using USP apparatus II (Dissolution system 2100B, Distek, New Jersey, USA) with a speed of 50 rpm. According to USP43-NF38 (The United States Pharmacopeial Convention Committee of Revision, 2021a), the simulated gastric fluid without pepsin, containing 2.0 g/L of sodium chloride and 7.0 mL/L of hydrochloric acid, was used as the medium. Each lyophilisate was placed in a vessel containing 900 mL of medium maintained at 37±0.5°C. At the suitable time interval, 5 mL aliquots of dissolution medium were withdrawn and replaced with an equal volume of fresh medium. The withdrawn samples were

filtrated through 0.45 µm syringe membrane filter, then determined with a UV-visible spectrophotometer (Evolution 200, Thermo Fisher Scientific, Massachusetts, USA) at a wavelength of 254 nm. The concentration of piroxicam was calculated with a calibration curve ranging from 10 to 40 µm/mL of piroxicam in a 0.01N methanolic hydrochloric acid solution and dissolution medium containing lyophilisate's excipients except piroxicam were used as spectrometry blank. The percentage of piroxicam released was then computed and a graph of cumulative drug dissolved against time was plotted. To compare the dissolution profiles, the model-independent mathematical approach employing the difference (f1) and similarity (f2) factors, defined by Equations (6) and (7), respectively, was used (Moore and Flanner, 1996; Shah et al., 1998).

$$f_1 = \left\{\frac{\sum|R_t - T_t|}{\sum R_t}\right\} \times 100 \quad (6)$$

$$f_2 = 50 \log\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2\right]^{-0.5} \times 100\right\} \quad (7)$$

when n is the number of sampling time, and R_t and T_t are the mean percentage dissolved at each time point, t, for reference and test dissolution prefile, respectively.

The f1 factor is proportional to the average difference while the f2 factor indicates the closeness between two dissolution profiles. Acceptable f1 values are between 0 and 15; therefore, f1 over 15 indicates significant dissimilarity. When the two profiles are identical, the f2 value equals 100. A f2 value of 50 or greater indicates similarity or equivalence of the curves of two dissolution profiles.

2.6 Sensory test

Twelve healthy volunteers participated in the sensory test. No subject reported having an allergy to piroxicam or excipients in lyophilized formulations. All subjects were nonsmokers and signed an informed consent before the experiment. Piroxicam/HPβCD lyophilisates with and without flavoring agents were dissolved and diluted with DI water to the final concentration of 0.1 mg/mL as the concentrations within the studied range from Yoshida's gustatory sensation test (Yoshida et al., 2014). The subjects were asked to gargle with DI water at least 250 mL before the test, keep the 5 mL of tested solution in their mouths for 10 sec and spit it out. Thereafter, subjects gargled with at least 250-mL DI water or until the bitterness of the previous sample disappeared. The degree of bitterness was classified, corresponding to increasing bitterness, as "not bitter", "almost not bitter", "slightly bitter" and "bitter". Because of the different personal perceptions of bitterness, all subjects were calibrated by receiving a piroxicam aqueous suspension (0.1 mg/mL) and designated as bitter. To avoid any suggestions, the study was performed single-blind, where only the examiner knew the composition of the solutions.

2.7 Data analysis

All results were presented as average±standard deviation (SD) of at least triplicate experiments. Statistical differences were analyzed by a t-test using Microsoft Excel. The differences were considered significant at 95% confident interval with a p-value less than 0.05.

3. RESULTS AND DISCUSSION

3.1 Phase-solubility profiles of piroxicam/βCD and piroxicam/HPβCD

Related studies showed that piroxicam tended to form inclusion complexes with various types of cyclodextrin, particularly βCD and its derivatives (Bertoluzza et al., 1999; Braibanti et al., 1998; Fronza et al., 1992; Mostafa et al., 2020; Zhang et al., 2009). According to Higuchi and Connors classification (Higuchi and Connors, 1965), the phase-solubility profiles of piroxicam/βCD and piroxicam/HPβCD in aqueous solution were A_L- and B_S type, respectively (Figure 3). The A_L-type indicated that increasing piroxicam solubility depended on the concentration of βCD. Moreover, the limitation of solubilization effect was observed in the B_S-type, phase-solubility profile of piroxicam/HPβCD. This result was uncorrelated with related reports where phase solubility presents the A_L-type (Doliwa et al., 2001; Jug and Bećirević-Laćan, 2004). It might have been due to the polymorphism properties of piroxicam. Sheth and coworkers reported the two polymorphs of piroxicam with differences in their crystal structure, molecular structure and color. Crystalline piroxicam exists as a colorless single crystals containing neutral piroxicam molecules. Another polymorph exists as yellow crystals containing zwitterionic piroxicam molecules (Sheth et al., 2004; Sheth et al., 2005). These zwitterion forms can exist in aqueous solutions (Bordner et al., 1989; Tsai et al., 1993) and exhibit lower solubility compared to the crystalline one (Paaver et al., 2012; Vrečer et al., 2003). If a phase transformation occurred during phase-solubility studies, the solubility of zwitterionic piroxicam was measured instead of the crystalline piroxicam. Notably, a molecular arrangement of zwitterionic piroxicam as dimers and tetramers through hydrogen bonding forms the inclusion complex with βCD, HPβCD, and methyl-βCD via the benzyl ring moiety (Rozou et al., 2004). Therefore, we assumed that the dimers and tetramers of the piroxicam zwitterion form an inclusion complex with HPβCD. The limit of aqueous solubility has been explained by completion of zwitterionic piroxicam in the high concentration of HPβCD (Loftsson and Brewster, 2010) resulted in Bs-type phase solubility.

In the linear region of the phase-solubility profiles, the $K_{1:1}$, CE, and piroxicam:CD ratios were calculated according to Equations (2)-(4) as shown in Table 1. The slope of phase solubility profile was less than unity, implying the formation of both piroxicam/βCD and piroxicam/HPβCD inclusion complexes with a 1:1 stoichiometry. This result of piroxicam/βCD was in good agreement with that reported in related literature (Banerjee et al., 2004; Braibanti et al., 1998) and was opposite the results of 1:2 from spectrofluorometric studies (Escandar, 1999; Xiliang et al., 2003). Interestingly, the stoichiometry of piroxicam/βCD depended on the solvent used to prepare the test solutions. The complex with a 1:2.5 ratio presented in water-acetonitrile (1:1, v/v) or anhydrous acetonitrile (Van Hees et al., 2002), and the 1:2 ratio was observed in nitric acid (HNO_3) (Escandar, 1999) or phosphate buffer (Xiliang et al., 2003). The CE of piroxicam/βCD was slightly higher than piroxicam/HPβCD complexes (0.021 and 0.018, respectively). These could be explained by the interference of hydroxypropyl group located outside the

HPβCD cavity. Also, the piroxicam:CD ratio implied that the required quantity of HPβCD was higher than βCD to increase the solubility of piroxicam corresponding to related literature (Loftsson and Brewster, 2010).

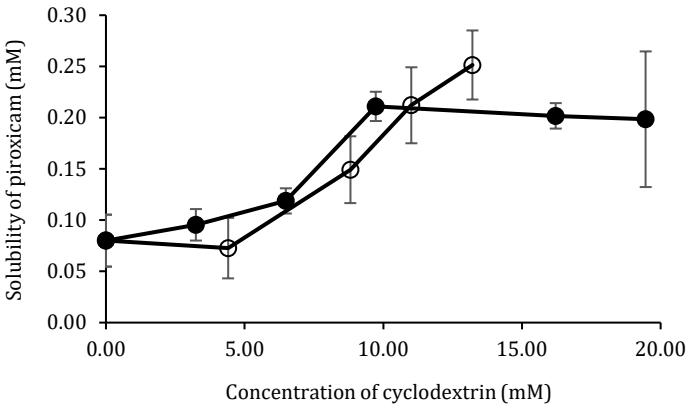

Figure 3. Phase solubility diagrams of piroxicam in aqueous βCD (○) or HPβCD (●) solutions at 25 ± 5°C

Table 1. Apparent stability constant ($K_{1:1}$), complexation efficiency (CE), and piroxicam:CD (P:CD) ratio of piroxicam/βCD and piroxicam/HPβCD complexes

Complexes	$K_{1:1}$ (M^{-1})	CE	P:CD ratio
Piroxicam/βCD	175.9±92.5	0.0211±0.005	1:48
Piroxicam/HPβCD	162.7±96.1	0.0181±0.003	1:56

3.2 Molecular docking

To understand the molecular recognition of both piroxicam/βCD and piroxicam/HPβCD complexes, molecular docking with a Lamarckian genetic algorithm was employed to identify appropriate binding modes and conformation of those complexes. The docking studies of piroxicam/βCD complex revealed that piroxicam aligned in the cavity of βCD with a binding energy equal to 7.51 kJ/mol. The molecular interaction of piroxicam/βCD complexes showed that the pyridine moiety occupied the hydrophobic cavity of the βCD facing the primary face, i.e., narrow rim. The benzene ring was oriented toward the narrow rim while the benzothiazine ring was located near the wide rim (Figure 4(a) and 4(b)). Moreover, the most stable conformation of the piroxicam/HPβCD complex was suggested to be the partial inclusion complex with a binding energy of 7.50 kJ/mol. Due to the steric hindrance of hydroxypropyl groups located at the narrow rim of the cavity, benzene, and pyridine moieties were included near the secondary face while the benzothiazine ring was located outside the HPβCD's cavity (Figure 4(c) and 4(d)). From the docking results, the molecular arrangements of piroxicam/βCD complexes are in good agreement with the NMR data and related studies (Fronza et al., 1992; Raffaini and Ganazzoli, 2020).

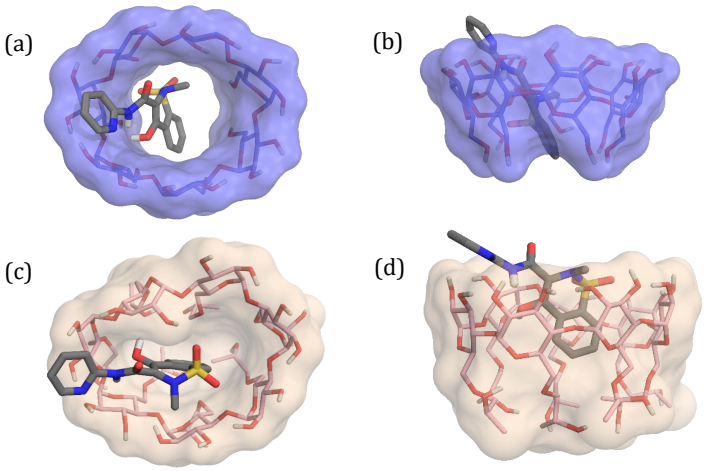

Figure 4. The top (left) and side view (right) of the piroxicam/βCD (a) and (b) and piroxicam/HPβCD (c) and (d) 1:1 host-guest complexes in the most stable geometries. The piroxicam molecule was colored by atoms (color code: C atoms were gray, O atoms were red, N atoms were dark blue, and S atoms were yellow). The molecular surfaces of βCD and HPβCD were shown as purple and faded yellow, respectively, for clarity

3.3 Preparation of piroxicam lyophilisates

Formulation of blank lyophilisates was first carried out to determine a suitable polymer (Table 2). Regarding the effect of the polymer, lyophilisates of gelatin (F1) and HPMC (F2) were studied. Gelatin-based lyophilisate disintegrated in simulated saliva faster than the other one (5.58±1.25 and 21.87±1.27 sec, respectively). The HPMC-based lyophilisates disintegrated and completely dissolved in simulated saliva at the same time while a small fraction of gelatin first remained afterwards and slowly dissolved. The lyophilisates, consisting of various ratios of gelatin and HPMC, were then studied (F3-F7). Disintegration time of the obtained tablets decreased regarding the increment of HPMC concentration. Even though F7 showed the fastest disintegration time (17.31±5.46 sec), deformed lyophilisates were observed during the collection. Thus, the mixture ratio of 2:3 (F5) was selected for further investigation due to the fast disintegration time and good removability from the mold. Notably, the removability of lyophilisates depended on the material of mold. Due to the inflexibility of the polystyrene mold, deformed lyophilisates were easily obtained. Therefore, PEG400 was added as plasticizer to increase elasticity of piroxicam lyophilisates (F8-F10). The concentration of PEG400 affected the disintegration time and removability from the mold. Considering the disintegration time and removability, the F9 was the most suitable formulation for further studies.

According to the phase-solubility profiles of piroxicam/βCD and piroxicam/HPβCD (Figure 3), the 1.5% w/w of βCD and 2.5% w/w of HPβCD, showing the highest solubilizing effect on piroxicam, were selected to investigate the effect of CDs in piroxicam lyophilisates. The presence of CDs in piroxicam lyophilisates affected disintegration time (Figure 5). Incorporating either βCD or HPβCD in piroxicam lyophilisate decreased disintegration time approximately 1.7 and 2.1 times, respectively. Therefore, the formulation containing piroxicam/HPβCD was selected for flavoring with sweetener and acidifying agents.

Stevioside and erythritol were selected as investigated sweeteners as shown in Table 3. Both sweeteners showed slight effects on the disintegration time of piroxicam/HPβCD lyophilisates. Erythritol was selected as the sweetener due to its lower disintegration time. Increasing the quantity of erythritol did not affect the disintegration time, as shown in F9B and F9C. Because the addition of citric acid insignificantly influenced the disintegration time of the obtained tables (p-value >0.05 at a 95% confidence interval), F9D was finally chosen for dissolution studies and sensory test. Piroxicam and piroxicam/HPβCD lyophilisates (Figure 6) were prepared in accordance with the F9D formulation. It has shown that average weight of piroxicam/HPβCD lyophilisates (73.31±2.30 mg) was higher than piroxicam lyophilisates (48.61±1.76 mg) due to the high amount of HPβCD in the formulation.

Table 2. Composition of the aqueous polymer solution for investigating the effect of polymer and plasticizer on disintegration time (DT) and removability from mold

Formulation	Concentration (%w/w)				DT (sec)	Removability from the mold*
	Gelatin	HPMC	Piroxicam	PEG400		
F1	1	-	-	-	5.58±1.25	+
F2	-	1	-	-	21.87±1.27	++
F3	1	1	-	-	48.81±4.26	++
F4	0.4	1.6	-	-	26.50±3.30	++
F5	0.8	1.2	-	-	23.68±4.35	++
F6	1.2	0.8	-	-	31.98±7.68	+++
F7	1.6	0.4	-	-	17.31±5.46	+++
F8	0.8	1.2	1	0.1	33.66±6.14	+
F9	0.8	1.2	1	0.2	20.01±3.51	+
F10	0.8	1.2	1	0.3	31.24±3.81	+++

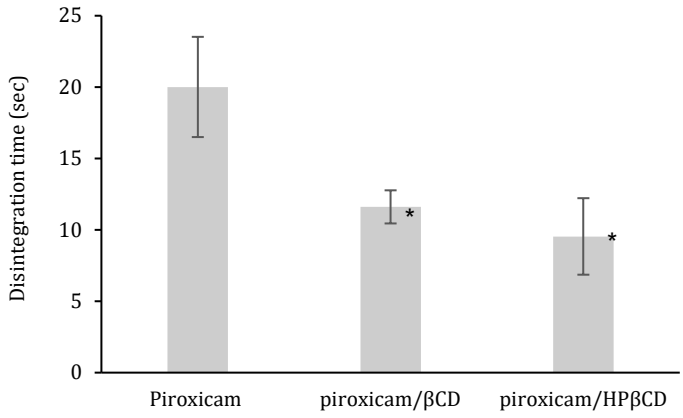

Figure 5. Disintegration time of piroxicam lyophilisates containing βCD or HPβCD. * indicate p-value < 0.05

Table 3. Effect of sweeteners and acidifying agent on disintegration time of piroxicam/HPβCD lyophilisates

Formulation	Concentration (%w/w)			Disintegration time (sec)
	Stevioside	Erythritol	Citric acid	
F9A	0.5	-	-	8.99±2.87
F9B	-	0.5	-	6.25±0.71
F9C	-	1	-	7.35±2.75
F9D	-	1	0.75	10.99±1.90

Figure 6. Image of piroxicam/HPβCD lyophilisates consisting of 0.8% w/w gelatin, 1.2% w/w HPMC, 1% w/w piroxicam, 2.5% w/w HPβCD, 0.2% w/w PEG400, 1% w/w erythritol, and 0.75% citric acid

3.4 Morphology of lyophilisates

Surface and cross-sectional SEM views, micrographs of blank (F9D without piroxicam), piroxicam (F9D without HPβCD), piroxicam/βCD (replaced HPβCD in F9D with βCD) and piroxicam/HPβCD lyophilisates (F9D) were determined. The presence of both CDs influenced the morphology of lyophilisate. The needle-like and polyhedron particles of βCD and HPβCD, respectively, were observed on the surface of the CD lyophilisate, while the surface of blank and piroxicam lyophilisates was nearly smooth (Figure 7).

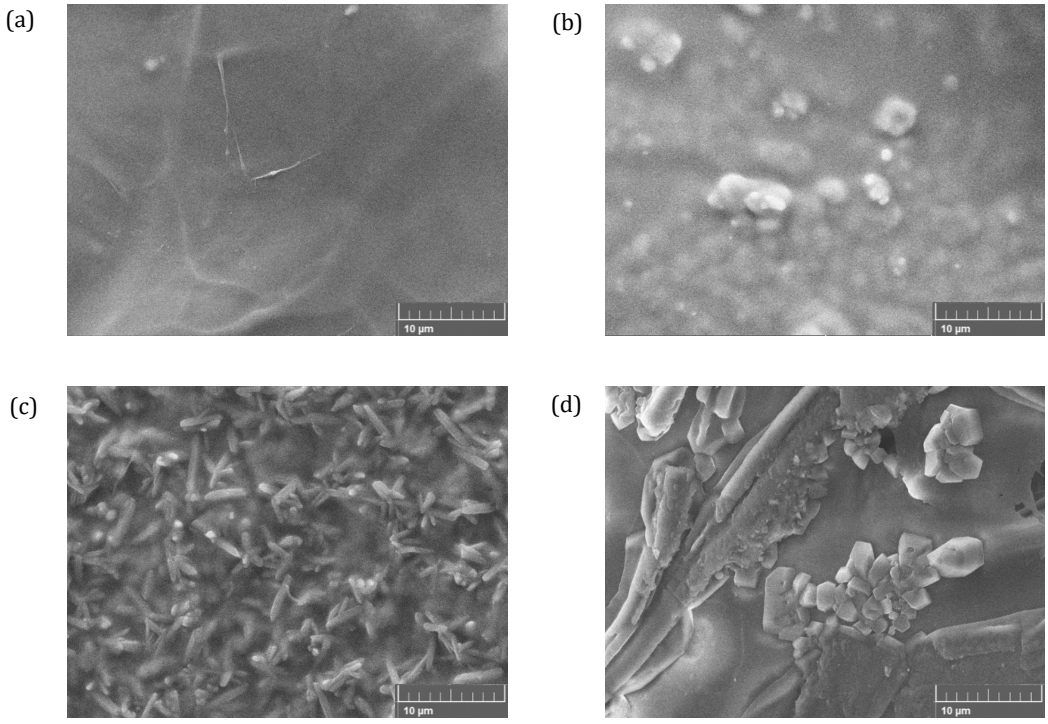

Figure 7. Surface view SEM micrographs of the blank (a), piroxicam (b), piroxicam/βCD (c) and piroxicam/HPβCD lyophilisates (d) at 50000×

3.5 ^{1}H NMR analysis

The ^{1}H NMR spectrometry was used to confirm the piroxicam/βCD and piroxicam/HPβCD complexation in the liquid-state. Due to the insufficient solubility of lyophilisates in D_2O, DMSO-d6 was used as the solvent. The signals of free compounds, i.e., piroxicam, βCD, and HPβCD, in DMSO-d6 and DMSO-d6 containing other excipients of lyophilisate (or composite solution), were assigned according to related studies (Kinal et al., 2019; Onnainty et al., 2011; Raoov et al., 2014; Silva et al., 2018). Therefore, the complexes information was obtained from the comparison of the proton chemical shifts (Δδ) observed from the piroxicam/CD mixture with those found from the individual compounds (Table 4). In the presence of piroxicam, the signals of H-3, H-5, and H-6 located inside the CD cavity showed unusual downfield shifts resulting from the summary of small upfield shifts of multiple signals (Liu et al., 2009).

Table 4. ^{1}H NMR chemical shifts (Δδ, ppm) of piroxicam, βCD, and HPβCD protons in DMSO-d6 and composite DMSO-d6 solutions

Assignment	Piroxicam/βCD		Piroxicam/HPβCD	
	DMSO-d6	Composite solution	DMSO-d6	Composite solution
H5' of pyridine ring	-0.0020	-0.0022	-0.0035	0.0064
H3' of pyridine ring	-0.0026	-0.0008	-0.0036	0.0009
H6' of pyridine ring	-0.0069	-0.0024	-0.0071	0.0035
Methyl group of benzothiazine ring	-0.0054	-0.0021	-0.0056	0.0025
H6 + H7 + H8 of benzene ring	-0.0071	-0.0026	-0.0070	0.0056
H5 of benzene ring	0.0003	-0.0003	-0.0019	-0.0073
H-3, H-5 and H-6 of βCD	0.0049	0.0058	-	-
H-1 of βCD	0.0023	0.0045	-	-
H-3 and H-6 of HPβCD	-	-	0.0007	0.0048

However, the ring current effect entering the CD cavity was observed through the chemical shifts of protons on piroxicam, which were also affected by the presence of CD. The upfield shift of the H5, H6, H7, and H8 of the benzene ring on piroxicam was prominent in DMSO-d6 and composite solutions indicating the benzene ring was shielded and included in the CD cavity (Zhao et al., 2011). The chemical shifts of protons on the benzene ring also implied that the molecular conformation of piroxicam/CD depended on the type of CD and composition of solvent, i.e., excipients of lyophilisate. The presence of lyophilisate compositions such as gelatin, HPMC, and PEG400 affected the formation of piroxicam/CD complexes. Notably, the conformation change of the piroxicam/CD complex was complicated due to the lyophilisate composition. Changing details could be elaborated on using two-dimensional NMR spectroscopy, e.g., rotating frame overhauser enhancement spectroscopy, ROSEY, together with molecular dynamic simulation.

Even though the chemical shifts of the benzothiazine ring in the piroxicam/HPβCD complex were remarkable, it could not be inserted in the hydrophobic cavity of HPβCD due to the hinderance of molecular geometry. Rozou et al. (2004) proposed that these signals could be attributed to the formation of the piroxicam dimer during the inclusion complex formation. Therefore, the inclusion complex of piroxicam/HPβCD was considered as a partial complex regarding the results from molecular docking (Figures 4(c) and 4(d)). Those downfield signals, especially piroxicam/HPβCD in composite solution, suggested multiple equilibria of complex formation (Fronza et al., 1992). Moreover, the dominant chemical shift of H5 on the benzene ring (-0.0073) in composite solution indicated the molecular conformational change of piroxicam/HPβCD complexes due to the presence of excipients.

3.6 Dissolution profiles of piroxicam/HPβCD lyophilisates

According to the attributes of lyophilisates, some APIs might dissolve in saliva, then become completely soluble in the gastrointestinal tract. The dissolution profiles of piroxicam from the lyophilisates in artificial saliva were preliminary investigated using the USP apparatus II with a speed of 50 rpm in 900-mL artificial saliva, pH 6.8. The results showed that the percentage of dissolved piroxicam in lyophilisates was higher than 90 within 5 min, then the dissolution profiles indicated a plateau (data not show). These might be due to the remarkable disintegration times of lyophilisates, the solubility improvement from lyophilization (Dixit and Kulkarni, 2012), the appropriation of the dissolution condition, especially in terms of medium volume. It was noted that the solubility of piroxicam is pH dependent (Shohin et al., 2014). It is an enolic acid molecule with a pKa of 6.3. The deprotonation constant in the excited state (pKa*) in both the presence and absence of βCD are 5.58 and 2.70, respectively, indicate that the enolic group in more acidic than in the ground state (Escandar, 1999). Therefore, in artificial saliva pH 6.8, piroxicam in the ionized form presented the high percentage of dissolution from lyophilisates within 5 min, Moreover, if the rapidly dissolving product shows the percentage of release higher than 85 within 15 min, a profile comparison is not required (The United States Pharmacopeial Convention Committee of Revision, 2021b). However, even though it revealed an extremely fast disintegration, when the whole of the lyophilisates were swallowed, the major drug release would occur in gastric fluid. According to NMR results (section 3.5), in the liquid state, piroxicam/HPβCD partially inclusion complexes also formed even in the presence of excipients. Therefore, the dissolution behaviors of the piroxicam in gastric condition were investigated (Figure 8).

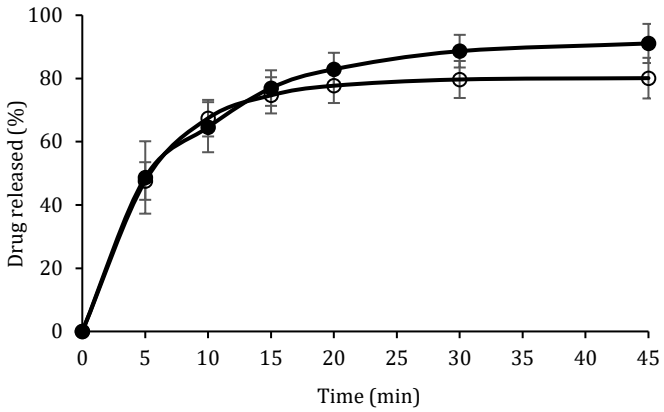

Figure 8. In vitro dissolution profiles for piroxicam lyophilisates (○) and piroxicam/HPβCD (●) lyophilisates in stimulated gastric fluid without pepsin

Piroxicam dissolution profiles from lyophilisates and HPβCD lyophilisates were compared employing the model-independent approach. The f1 and f2 values (7 and 61, respectively) indicated the similarity between two dissolution profiles. Even though the presence of HPβCD did not significantly affect the dissolution of piroxicam, a slight increase in of piroxicam from HPβCD lyophilisates after 15 minutes might be due to the solubilizing effect of those partial piroxicam/HPβCD inclusion complexes.

3.7 Sensory study

Like most nonsteroidal anti-inflammatory drugs, piroxicam possesses a bitter taste (Mostafa et al., 2020). Even though the perception of taste is subjective, the results from the bitterness evaluation (Table 5) showed some expected outliners. Since the 0.1 mg/mL piroxicam suspension was designated as bitter, it could imply that HPβCD in lyophilisates suppressed the perceived bitterness. Moreover, all subjects demonstrated the same overall pattern of bitterness for piroxicam/HPβCD lyophilisates with or without flavoring agents, i.e., erythritol, and citric acid. Four and five of the 12 subjects reported no bitterness or almost not bitter, respectively. It was assumed that flavoring agents in lyophilisates did not play an important role in bitter suppression. Arima and coworkers proposed that CD masks the bitter taste of drugs by directly interacting with bitter taste receptors (T2Rs) of the taste buds (Arima et al., 2012). Various studies have reported that CD, especially derivatives of βCD, showed the ability to extract cholesterol or phospholipids, resulting in protein removal from the cell membrane (Arima et al., 2001; Arima et al., 2004; Yunomae et al., 2003). Therefore, HPβCD may decrease the function of G protein-coupled receptors localized in the lipid membrane of taste cells through the extraction of cholesterol and phospholipids, showing the taste-masking effect of piroxicam. Notably, the concentration of flavoring agents used in the formulation might be insufficient for bitter taste-masking.

Table 5. Evaluation of the bitterness of piroxicam/HPβCD lyophilisates by the healthy volunteers

Piroxicam/HPβCD lyophilisate	Number of volunteers designating the bitterness			
	Not bitter	Almost not bitter	Slightly bitter	Bitter
Piroxicam suspension (control)	-	-	-	12
Without flavoring agent	4	5	3	0
With flavoring agent	4	5	2	1

4. CONCLUSION

In phase-solubility studies, the inclusion complexes of piroxicam with βCD and HPβCD presented a similar stability constant, i.e., $K_{1:1}$, but the quantity of HPβCD used in formulation should be higher due to the higher drug: CD ratio. Compared with one polymer, using a mixture of gelatin and HPMC in a 2:3 ratio showed the most favorable combination in respect to disintegration time. The morphology of piroxicam/CD lyophilisates relied on the presence and type of CD. Additionally, molecular conformation of piroxicam/CD inclusion complexes depended on the type of CD and the presence of excipients. The inclusion complex formation with multiple equilibria was observed for piroxicam/HPβCD in the presence of excipients. Furthermore, HPβCD in piroxicam lyophilisates suppressed the perceived bitterness reported by volunteers. Fast-dissolving piroxicam tablets containing HPβCD, were successfully prepared using the lyophilization technique.

ACKNOWLEDGMENT

The authors would like to express their gratitude to Solutrade Co., Ltd., Bangkok, Thailand, for kindly providing samples and staff at the Department of Manufacturing Pharmacy, College of Pharmacy, Rangsit University. We are also immensely grateful to Nattharat Chiengthong, Natsuda Jiamjitvanich, and Thanchanok Wiriyatangsakul for their diligence and hard work.

REFERENCES

Alcaro, S., Ventura, C. A., Paolino, D., Battaglia, D., Ortuso, F., Cattel, L., Puglisi, G., and Fresta, M. (2002). Preparation, characterization, molecular modeling and in vitro activity of paclitaxel–cyclodextrin complexes. *Bioorganic & Medicinal Chemistry Letters,* 12(12), 1637–1641.

AlHusban, F., Perrie, Y., and Mohammed, A. R. (2010). Formulation and characterisation of lyophilised rapid disintegrating tablets using amino acids as matrix forming agents. *European Journal of Pharmaceutics and Biopharmaceutics,* 75(2), 254–262.

Arima, H., Higashi, T., and Motoyama, K. (2012). Improvement of the bitter taste of drugs by complexation with cyclodextrins: Applications, evaluations and mechanisms. *Therapeutic Delivery,* 3(5), 633–644.

Arima, H., Yunomae, K., Hirayama, F., and Uekama, K. (2001). Contribution of P-glycoprotein to the enhancing effects of dimethyl-β-cyclodextrin on oral bioavailability of tacrolimus. *Journal of Pharmacology and Experimental Therapeutics,* 297(2), 547–555.

Arima, H., Yunomae, K., Morikawa, T., Hirayama, F., and Uekama, K. (2004). Contribution of cholesterol and phospholipids to inhibitory effect of dimethyl-beta-cyclodextrin on efflux function of P-glycoprotein and multidrug resistance-associated protein 2 in vinblastine-resistant Caco-2 cell monolayers. *Pharmaceutical Research,* 21(4), 625–634.

Banchero, M., and Manna, L. (2011). Investigation of the piroxicam/hydroxypropyl-β-cyclodextrin inclusion complexation by means of a supercritical solvent in the presence of auxiliary agents. *The Journal of Supercritical Fluids,* 57(3), 259–266.

Banerjee, R., Chakraborty, H., and Sarkar, M. (2004). Host–guest complexation of oxicam NSAIDs with β-cyclodextrin. *Biopolymers,* 75(4), 355–365.

Bertoluzza, A., Rossi, M., Taddei, P., Redenti, E., Zanol, M., and Ventura, P. (1999). FT-Raman and FT-IR studies of 1:2.5 piroxicam: β-cyclodextrin inclusion compound. *Journal of Molecular Structure,* 480–481, 535–539.

Boateng, J. S., Matthews, K. H., Auffret, A. D., Humphrey, M. J., Eccleston, G. M., and Stevens, H. N. (2012). Comparison of the in vitro release characteristics of mucosal freeze-dried wafers and solvent-cast films containing an insoluble drug. *Drug Development and Industrial Pharmacy,* 38(1), 47–54.

Bordner, J., Hammen, P. D., and Whipple, E. B. (1989). Deuterium isotope effects on carbon-13 NMR shifts and the tautomeric equilibrium in N-substituted pyridyl derivatives of piroxicam. *Journal of the American Chemical Society,* 111(17), 6572–6578.

Bouchal, F., Skiba, M., Chaffai, N., Hallouard, F., Fatmi, S., and Lahiani-Skiba, M. (2015). Fast dissolving cyclodextrin complex of *piroxicam* in solid dispersion Part I: Influence of β-CD and HPβ-CD on the dissolution rate of *piroxicam*. *International Journal of Pharmaceutics,* 478(2), 625–632.

Braibanti, A., Fisicaro, E., Ghiozzi, A., Compari, C., and Bovis, G. (1998). Host-guest interactions between β-cyclodextrin and piroxicam. *Reactive and Functional Polymers,* 36(3), 251–255.

Chandrasekhar, R., Hassan, Z., AlHusban, F., Smith, A. M., and Mohammed, A. R. (2009). The role of formulation excipients in the development of lyophilised fast-disintegrating tablets. *European Journal of Pharmaceutics and Biopharmaceutics,* 72(1), 119–129.

Ciper, M., and Bodmeier, R. (2006). Modified conventional hard gelatin capsules as fast disintegrating dosage form in the oral cavity. *European Journal of Pharmaceutics and Biopharmaceutics,* 62(2), 178–184.

Dassault Systèmes. (2021). BIOVIA Discovery Studio. *Dassault Syst mes BIOVIA, Discovery Studio Modeling Environment*. [Online URL: https://www.3ds.com/products-services/biovia/resource-center/citations-and-references/] accessed on May 10, 2021.

De Sousa, F. B., Denadai, Â. M. L., Lula, I. S., Lopes, J. F., Dos Santos, H. F., De Almeida, W. B., and Sinisterra, R. D. (2008). Supramolecular complex of fluoxetine with β-cyclodextrin: An experimental and theoretical study. *International Journal of Pharmaceutics,* 353(1–2), 160–169.

Dixit, M., and Kulkarni, P. K. (2012). Lyophilization monophase solution technique for improvement of the solubility and dissolution of piroxicam. *Research in Pharmaceutical Sciences,* 7(1), 13–21.

Doliwa, A., Santoyo, S., and Ygartua, P. (2001). Influence of piroxicam: Hydroxypropyl-beta-cyclodextrin complexation on the in vitro permeation and skin retention of piroxicam. *Skin Pharmacology and Applied Skin Physiology,* 14(2), 97–107.

Escandar, G. M. (1999). Spectrofluorimetric determination of piroxicam in the presence and absence of beta-cyclodextrin. *Analyst,* 124(4), 587–591.

Farias, S., and Boateng, J. S. (2018). Development and functional characterization of composite freeze dried wafers for potential delivery of low dose aspirin for elderly people with dysphagia. *International Journal of Pharmaceutics,* 553(1–2), 65–83.

Fronza, G., Mele, A., Redenti, E., and Ventura, P. (1992). Proton nuclear magnetic resonance spectroscopy studies of the inclusion complex of piroxicam with *β*-cyclodextrin. *Journal of Pharmaceutical Sciences,* 81(12), 1162–1165.

Han, D., Han, Z., Liu, L., Wang, Y., Xin, S., Zhang, H., and Yu, Z. (2020). Solubility enhancement of myricetin by inclusion complexation with heptakis-*O*-(2-hydroxypropyl)-β-cyclodextrin: A joint experimental and theoretical study. *International Journal of Molecular Sciences,* 21(3), 766.

Higuchi, T., and Connors, K. A. (1965). Phase-solubility techniques. *Advances in Analytical Chemistry of Instrumentation,* 4, 117–212.

Inoue, Y. (1993). NMR studies of the structure and properties of cyclodextrins and their inclusion complexes. In *Annual Reports on NMR Spectroscopy Vol. 27* (Webb, G. A., Ed.), pp. 59–101. Cambridge: Academic Press.

Iurian, S., Bogdan, C., Tomuță, I., Szabó-Révész, P., Chvatal, A., Leucuța, S. E., Moldovan, M., and Ambrus, R. (2017). Development of oral lyophilisates containing meloxicam nanocrystals using QbD approach. *European Journal of Pharmaceutical Sciences,* 104, 356–365.

Jug, M., and Bećirević-Laćan, M. (2004). Influence of hydroxypropyl-β-cyclodextrin complexation on piroxicam release from buccoadhesive tablets. *European Journal of Pharmaceutical Sciences,* 21(2–3), 251–260.

Kianfar, F., Ayensu, I., and Boateng, J. S. (2014). Development and physico-mechanical characterization of carrageenan and poloxamer-based lyophilized matrix as a potential buccal drug delivery system. *Drug Development and Industrial Pharmacy,* 40(3), 361–369.

Kinal, A., Güreşci, M., AlRrabiah, H., Abdel-Aziz, H. A., and Mostafa, G. A. E. (2019). Synthesis, spectroscopic

characterization and structural investigation of new charge-transfer complexes of piroxicam with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone and chloranilic acid: Experimental and theoretical studies. *Materials Express,* 9(3), 203–212.

Liu, J., Jiang, N., Ma, J., and Du, X. (2009). Insight into unusual downfield NMR shifts in the inclusion complex of acridine orange with cucurbit[7]uril. *European Journal of Organic Chemistry,* 2009(29), 4931–4938.

Loftsson, T., and Brewster, M. E. (2010). Pharmaceutical applications of cyclodextrins: Basic science and product development. *Journal of Pharmacy and Pharmacology,* 62(11), 1607–1621.

Loftsson, T., Hreinsdóttir, D., and Másson, M. (2005). Evaluation of cyclodextrin solubilization of drugs. *International Journal of Pharmaceutics,* 302(1–2), 18–28.

Loftsson, T., Hreinsdóttir, D., and Másson, M. (2007). The complexation efficiency. *Journal of Inclusion Phenomena and Macrocyclic Chemistry,* 57(1), 545–552.

Madi, F., Khatmi, D., Dhaoui, N., Bouzitouna, A., Abdaoui, M., and Boucekkine, A. (2009). Molecular model of CENS piperidine β-CD inclusion complex: DFT study. *Comptes Rendus Chimie,* 12(12), 1305–1312.

Marques, M. R. C., Loebenberg, R., and Almukainzi, M. (2011). Simulated biological fluids with possible application in dissolution testing. *Dissolution Technologies,* 18(3), 15–28.

Mirza, S., Miroshnyk, I., Habib, M. J., Brausch, J. F., and Hussain, M. D. (2010). Enhanced dissolution and oral bioavailability of piroxicam formulations: Modulating effect of phospholipids. *Pharmaceutics,* 2(4), 339–350.

Mizumoto, T., Masuda, Y., Yamamoto, T., Yonemochi, E., and Terada, K. (2005). Formulation design of a novel fast-disintegrating tablet. *International Journal of Pharmaceutics,* 306(1–2), 83–90.

Moore, J. W., and Flanner, H. H. (1996). Mathematical comparison of dissolution profiles. *Pharmaceutical Technology,* 20(6), 64–74.

Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., and Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry,* 30(16), 2785–2791.

Mostafa, G. A. E., Al-Dosseri, A. S., and Al-Badr, A. A. (2020). Chapter Seven - Piroxicam. In *Profiles of Drug Substances, Excipients and Related Methodology, Vol. 45* (Brittain, H. G., Ed.), pp. 199–474. Cambridge: Academic Press.

Mura, P. A., Mennini, N., Kosalec, I., Furlanetto, S., Orlandini, S., and Jug, M. (2015). Amidated pectin-based wafers for econazole buccal delivery: Formulation optimization and antimicrobial efficacy estimation. *Carbohydrate Polymers,* 121, 231–240.

Onnainty, R., Longhi, M. R., and Granero, G. E. (2011). Complex formation of chlorhexidine gluconate with hydroxypropyl-β-cyclodextrin (HPβCD) by proton nuclear magnetic resonance spectroscopy (^{1}H NMR). *Carbohydrate Research,* 346(8), 1037–1046.

Paaver, U., Lust, A., Mirza, S., Rantanen, J., Veski, P., Heinämäki, J., and Kogermann, K. (2012). Insight into the solubility and dissolution behavior of piroxicam anhydrate and monohydrate forms. *International Journal of Pharmaceutics,* 431(1–2), 111–119.

Parkash, V., Maan, S., Deepika, Yadav, S. K., Hemlata, and Jogpal, V. (2011). Fast disintegrating tablets: Opportunity in drug delivery system. *Journal of Advanced Pharmaceutical Technology & Research,* 2(4), 223–235.

Polekhina, G., Gupta, A., van Denderen, B. J. W., Feil, S. C., Kemp, B. E., Stapleton, D., and Parker, M. W. (2005). Structural basis for glycogen recognition by AMP-activated protein kinase. *Structure,* 13(10), 1453–1462.

Raffaini, G., and Ganazzoli, F. (2020). Understanding surface interaction and inclusion complexes between piroxicam and native or crosslinked β-cyclodextrins: The role of drug concentration. *Molecules,* 25(12), 2848.

Raoov, M., Mohamad, S., and Abas, M. R. (2014). Synthesis and characterization of β-cyclodextrin functionalized ionic liquid polymer as a macroporous material for the removal of phenols and As(V). *International Journal of Molecular Sciences,* 15(1), 100–119.

Redenti, E., Zanol, M., Ventura, P., Fronza, G., Comotti, A., Taddei, P., and Bertoluzza, A. (1999). Raman and solid state 13C-NMR investigation of the structure of the 1 : 1 amorphous piroxicam : β-cyclodextrin inclusion compound. *Biospectroscopy,* 5(4), 243–251.

Rozou, S., Voulgari, A., and Antoniadou-Vyza, E. (2004). The effect of pH dependent molecular conformation and dimerization phenomena of piroxicam on the drug:cyclodextrin complex stoichiometry and its chromatographic behaviour: A new specific HPLC method for piroxicam:cyclodextrin formulations. *European Journal of Pharmaceutical Sciences,* 21(5), 661–669.

Scarpignato, C. (2013). Piroxicam-β-cyclodextrin: A GI safer piroxicam. *Current Medicinal Chemistry,* 20(19), 2415–2437.

Schrödinger. (2021). PyMOL. *The PyMOL Molecular Graphics System (Version 2.0)*. [Online URL: https://pymol.org/2/ support.html?] accessed on May 10, 2021.

Seager, H. (1998). Drug-delivery products and the Zydis fast-dissolving dosage form. *Journal of Pharmacy and Pharmacology,* 50(4), 375–382.

Shah, V. P., Tsong, Y., Sathe, P., and Liu, J.-P. (1998). In vitro dissolution profile comparison—statistics and analysis of the similarity factor, f2. *Pharmaceutical Research,* 15(6), 889–896.

Sheth, A. R., Bates, S., Muller, F. X., and Grant, D. J. W. (2004). Polymorphism in piroxicam. *Crystal Growth & Design,* 4(6), 1091–1098.

Sheth, A. R., Lubach, J. W., Munson, E. J., Muller, F. X., and Grant, D. J. W. (2005). Mechanochromism of piroxicam accompanied by intermolecular proton transfer probed by spectroscopic methods and solid-phase changes. *Journal of the American Chemical Society,* 127(18), 6641–6651.

Shohin, I. E., Kulinich, J. I., Ramenskaya, G. V., Abrahamsson, B., Kopp, S., Langguth, P., Polli, J. E., Shah, V. P., Groot, D. W., Barends, D. M., and Dressman, J. B. (2014). Biowaiver monographs for immediate release solid oral dosage forms: Piroxicam. *Journal of Pharmaceutical Sciences,* 103(2), 367–377.

Silva, M. R. M., Santos, E. P., Barros, R. C. S. A., Garcia, S., Albuquerque, M. G., Oliveira, J. S. C., and Sader, M. S. (2018). The development of a new complexation technique of hydrocortisone acetate with 2-Hydroxypropyl-β-cyclodextrin: Preparation and characterization. *Journal of Analytical & Pharmaceutical Research,* 7(1), 00194.

Snor, W., Liedl, E., Weiss-Greiler, P., Viernstein, H., and Wolschann, P. (2009). Density functional calculations on meloxicam–β-cyclodextrin inclusion complexes. *International Journal of Pharmaceutics,* 381(2), 146–152.

The United States Pharmacopeial Convention Committee of Revision. (2021a). *Piroxicam Capsules*. USP-NF Online. [Online URL: https://online.uspnf.com/uspnf/document/1_GUID-BB4CBA15-307B-452A-A6B7-30C1F5C9461D_2_en-US?source=Search%20Results&highlight=piroxicam] accessed on November 24, 2022.

The United States Pharmacopeial Convention Committee of Revision. (2021b). *The Dissolution Procedure: Development and Validation*. USP-NF Online. [Online URL: https://online.uspnf.com/uspnf/document/1_GUIDAC788D41-90A2-4F36-A6E7769954A9ED09_3_enUS?source=Quick%20Search&highlight=dissolution] accessed on November 24, 2022.

Topuz, F. (2022). Rapid sublingual delivery of piroxicam from electrospun cyclodextrin inclusion complex nanofibers. *ACS Omega,* 7(39), 35083–35091.

Tsai, R.-S., Carrupt, P.-A., Tayar, N. E., Giroud, Y., Andrade, P., Testa, B., Brée, F., and Tillement, J. P. (1993). Physicochemical and structural properties of non-steroidal anti-inflammatory oxicams. *Helvetica Chimica Acta,* 76(2), 842–854.

Van Hees, T., Piel, G., de Hassonville, S. H., Evrard, B., and Delattre, L. (2002). Determination of the free/included piroxicam ratio in cyclodextrin complexes: Comparison between UV spectrophotometry and differential scanning calorimetry. *European Journal of Pharmaceutical Sciences,* 15(4), 347–353.

Vrečer, F., Vrbinc, M., and Meden, A. (2003). Characterization of piroxicam crystal modifications. *International Journal of Pharmaceutics,* 256(1–2), 3–15.

Xiliang, G., Yu, Y., Guoyan, Z., Guomei, Z., Jianbin, C., and Shaomin, S. (2003). Study on inclusion interaction of piroxicam with β-cyclodextrin derivatives. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy,* 59(14), 3379–3386.

Yoshida, M., Haraguchi, T., and Uchida, T. (2014). Bitterness evaluation of acidic pharmaceutical substances (NSAIDs) using a taste sensor. *Chemical and Pharmaceutical Bulletin,* 62(12), 1252–1258.

Yunomae, K., Arima, H., Hirayama, F., and Uekama, K. (2003). Involvement of cholesterol in the inhibitory effect of dimethyl-beta-cyclodextrin on P-glycoprotein and MRP2 function in Caco-2 cells. *Federation of European Biochemical Societies,* 536(1–3), 225–231.

Zhang, X., Wu, D., Lai, J., Lu, Y., Yin, Z., and Wu, W. (2009). Piroxicam/2-hydroxypropyl-β-cyclodextrin inclusion complex prepared by a new fluid-bed coating technique. *Journal of Pharmaceutical Sciences,* 98(2), 665–675.

Zhao, R., Tan, T., and Sandström, C. (2011). NMR studies on Puerarin and its interaction with beta-cyclodextrin. *Journal of Biological Physics,* 37(4), 387–400.