ORIGINAL ARTICLE

Preparation and Evaluation of Domperidone/β-Cyclodextrin/Citric Acid/ Mannitol Quaternary Inclusion Complex: An *In Vitro* Study

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Abstract

Aim: The aim of this study is to prepare quaternary inclusion complex (QIC) comprised of domperidone (DOM), β-cyclodextrin (βCD), citric acid (CA), and mannitol and to evaluate its efficiency to enhance the solubility and dissolution rate of DOM. **Materials and Methods:** The phase solubility studies were conducted in water and CA solutions (10 mM) in the presence and absence of mannitol (1%). The physical mixtures and kneaded complexes of DOM/βCD, DOM/βCD/CA, and DOM/βCD/CA/mannitol were prepared for comparative study. The prepared mixtures and complexes were subjected to saturation solubility study in water and dissolution studies in 0.1 N HCl and phosphate buffer (pH 6.8). They were characterized by attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy, differential scanning calorimetry, X-ray powder diffraction, and scanning electron microscopy. **Results and Discussion:** It was found that mannitol and CA showed synergistic effect on the solubilizing efficiency of βCD. The QIC showed maximum solubility of DOM (819.2 ± 1.24 μg/ml) as compared to other systems. The dissolution studies revealed that the QICs showed maximum percentage dissolution efficiency and lowest mean dissolution time. The physicochemical characterization suggested reduction in crystallinity of DOM due to complexation and a non-covalent interaction of mannitol with other components of QIC. **Conclusion**: Overall study revealed that the QIC containing CA and mannitol is a promising alternative to other multicomponent systems and suitable for preparing orodispersible or sustained release dosage form of poorly soluble weakly basic drugs.

Key words: β-cyclodextrin, citric acid, domperidone, mannitol, multicomponent inclusion complex, solubility

INTRODUCTION

Domperidone (DOM), $C_{22}H_{24}ClN_5O_2$, is an antiemetic and prokinetic agent used in the treatment of nausea and vomiting for decades.[1,2] It has also been used for the treatment of migraine,[3,4] gastroparesis,[5] and functional dyspepsia.[1] DOM is structurally related to butyrophenones. The antiemetic properties of DOM may be attributed to its dopamine (D2) receptor-blocking activity in chemoreceptor trigger zone and at gastric level. As a prokinetic agent, DOM increases esophageal and gastric peristalsis and improves antroduodenal coordination which facilitates gastric emptying.[6,7] It is administered orally in the dose range of 10-40 mg daily and has elimination half-life of 5-7 h.[1] The short half-life of DOM necessitates its frequent administration in the disorders mentioned above which further reduces the patient compliance.

Till date, various approaches have been implemented to enhance the solubility of DOM including solid dispersions,[8] coevaporation,[9] and cyclodextrin complexation.[10] Cyclodextrins (CDs) are cyclic oligosaccharides which have been used in pharmaceutical industry as complexing agents to increase the aqueous solubility of poorly soluble drugs

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and to increase their bioavailability and stability.[11] The three-dimensional structure of CDs imparts them with the properties that are useful for pharmaceutical applications. CD molecules are versatile in having a hydrophobic cavity of size suitably enough to accommodate the lipophilic drugs as guests. The large number of hydroxyl groups on CDs renders them water-soluble. However, the natural CDs, specifically β-CDs (βCDs), exhibit low water solubility (18 mg/ml) due to the formation of internal hydrogen bond network in between secondary hydroxyl groups and relatively high crystal lattice energy. Moreover, the addition of βCD to the aqueous drug solutions or suspensions often results in the precipitation of the corresponding CD complexes.

To improve the aqueous solubility of βCD, various derivatives have been synthesized by modifying the hydrogen bond forming hydroxyl group of βCD.[12,13] Due to several reasons such as toxicity, cost, and dosage of βCD and its derivatives, the amount of CD to be used in most drug formulations should be limited.[14,15] The addition of hydroxy carboxylic acids,[16] hydrophilic polymers,[17] or amino acids,[18] during complexation between CDs and poorly soluble drugs, yields freely water-soluble complexes. The resultant multicomponent inclusion complexes dissolve very rapidly and give rise to supersaturated solutions that remain stable for several days.

In our previous study, it has been reported that the βCD alone increases the solubility of DOM by 2.2-fold whereas multicomponent inclusion complex comprising DOM, βCD, and citric acid (CA) increases the solubility of DOM by 76-fold.[19] Ribeiro *et al*. have reported that the quaternary inclusion complex (QIC) of a weakly basic drugs such as vinpocetine, βCD, tartaric acid, and water-soluble polymers enhances the solubility of vinpocetine to a greater extent than the ternary complex involving vinpocetine, βCD, and tartaric acid.[20] It was found that the polymers increased the stability constant of the ternary complex by co-complex formation. Mannitol has been investigated as a suitable candidate for enhancing the solubility of poorly soluble drugs due to its hydrotropic nature.[21,22] Basalious *et al*., 2014, have studied the effect of mannitol and CA on solubilizing property of βCD when used together in a fast-dissolving tablet; however, a detailed study including preparation of multicomponent inclusion complex and its characterization has not been performed.[23] In the present investigation, authors have prepared QIC comprised DOM, βCD, CA, and mannitol and studied the effect of mannitol on solubilizing efficiency of βCD in the presence of CA. The QICs were prepared by kneading method as it is simple and high yielding method. As DOM exhibits pH-dependent solubility, the effect of pH on dissolution of the prepared complexes was studied. The formation of inclusion complex was studied by Fourier transform infrared (FTIR), differential scanning calorimetry (DSC), X-ray powder diffraction (XRD), and scanning electron microscopy (SEM). The solubility, dissolution, and physicochemical properties of QICs were compared with DOM/βCD and DOM/βCD/CA systems.

MATERIALS AND METHODS

Materials

DOM was obtained as a gift sample from Vasudha Pharma Chem. Ltd. (Hyderabad, Telangana, India); βCD, mannitol, and CA were purchased from Loba Chemie (Mumbai, Maharashtra, India).

Phase solubility studies

The phase solubility studies for DOM were performed in distilled water and mannitol solution (1%) in the absence and presence of CA (10 mM).[16,24] Excess amount of DOM was weighed and added to the vials filled with distilled water (with and without CA) and mannitol solution (with and without CA) containing various concentrations of βCD in the range of 0-0.01 M. The solutions were shaken for 72 h at 37°C on a lab shaker (Bio-Technics, India). The specified volumes of samples were withdrawn and filtered through membrane filter (0.45 µm), diluted, and analyzed in a ultraviolet (UV)-visible spectrophotometer (Shimadzu, PharmSpec UV 1700, Japan) at 284 nm. Solubility measurements were performed in triplicate.

Preparation of inclusion complexes of DOM

Preparation of physical mixtures

DOM, βCD, and CA were weighed in an equimolar ratio (1:1:1) whereas the amount of mannitol was varied in different molar ratios (0.25, 0.5, and 1) with respect to other components. The weighed components were mixed and were pulverized using mortar and pestle followed by sifting through 250 µm mesh. A binary mixture comprised DOM/βCD (1:1) and a ternary mixture comprised DOM/βCD/CA (1:1:1) were also prepared for comparative study. The prepared mixtures were stored in airtight glass desiccators under vacuum.

Preparation of QICs by kneading

The QICs of DOM, βCD, CA, and mannitol, in the same molar ratio to that of PMs, were prepared by kneading method.[25] Accurately weighed quantity of βCD was mixed with sufficient quantity of water to obtain a smooth and homogeneous paste. Weighed quantity of DOM along with CA and mannitol was added slowly by grinding. The mixture was grounded for one hour. Finally, the paste was dried in hot air oven at 40°C for 48 h. The dried complex was powdered and passed through 250 µm mesh and stored in airtight glass

desiccators under vacuum till further use. A binary complex of DOM and βCD in molar ratio of 1:1 and ternary complex of DOM, βCD, and CA in molar ratio of 1:1:1 were prepared similarly for the comparative study.

Drug content estimation of inclusion complexes

QICs equivalent to 10 mg of DOM was accurately weighed and added into 100 ml volumetric flask and then 50 ml of 0.1 M HCl (methanolic) was added to it. The resultant solution was stirred for 60 min, till the entire drug leached out. The solution was filtered and suitably diluted with distilled water. Drug content was estimated spectrophotometrically at 284 nm using distilled water as blank. The same procedure was followed to determine the drug content in the physical mixtures and other kneaded complexes.

Saturation solubility studies

Saturation solubility studies were conducted for the physical mixtures, kneaded complexes, and the pure DOM in distilled water according to the method reported by Higuchi and Connors. Excesses of pure DOM, physical mixtures, and kneaded complexes were added to 20 ml distilled water taken in vials. The vials were shaken for 24 h using lab shaker at 37°C. After the complete equilibration, the supernatant solutions were collected carefully and filtered using membrane filter (0.45 μm). The concentration of DOM in filtered solution was determined using UV-visible spectrophotometer at 284 nm.

Dissolution studies

Dissolution studies of the physical mixtures, kneaded complexes, and pure DOM were performed in 900 ml, 0.1 N HCl and buffer solution, pH 6.8, using USP II type dissolution apparatus (Electrolab TDT-061, Mumbai, Maharashtra, India) at a stirring speed of 100 rpm. Powder sample equivalent to 30 mg of drug was clamped between infusion filter paper and immersed in the dissolution medium. An aliquot of 5 ml was withdrawn at predetermined intervals and filtered. Sink condition was maintained. Filtrates were assayed spectrophotometrically at 284 nm. The study was conducted in triplicate.

Further, the release data were subjected to data analysis to calculate various dissolution parameters, namely, mean dissolution time (MDT) and percentage dissolution efficiency (%DE).[26-30]

Statistical analysis

The results obtained from saturation solubility and dissolution studies were statistically validated by performing ANOVA using GraphPad Prism 6.0 software.

Characterization of inclusion complexes

Attenuated total reflectance-FTIR spectroscopy

The infrared spectra of DOM, βCD, CA, mannitol, physical mixtures (with equimolar ratios), and kneaded complexes (with equimolar ratios) were obtained using attenuated total reflectance (ATR)-FTIR spectrophotometer (Shimadzu, IR Affinity, Japan). The samples to be analyzed were transferred to the ATR compartment. The spectra were obtained for the range of 600-4000/cm at an average of 25 scans and resolution of 4/cm.

DSC

The DSC analysis was carried out using SDT Q600 V20.9 Build 20. The thermal behavior was studied by heating all samples (5-10 mg of drug or its equivalent complexes) in sealed aluminum pans, using an empty sealed pan as reference at the rate 10°C/min from 25 to 500°C under nitrogen atmosphere (flow rate: 10 ml/min).

XRD

X-ray diffraction patterns were recorded using X-ray diffractometer (PW1729, Philips, The Netherlands) with a copper target, operated at voltage of 30 kV, 30 mA current, at 2°C/min scanning speed and scanning angle ranging from 0 to 50° (2θ).

SEM

The surface morphology of DOM and kneaded complexes was studied using SEM, JEOL 6386®, Japan. Samples were sprinkled on to double-sided tape, sputter coated with platinum and examined in the microscope at 10 kV.

RESULTS AND DISCUSSION

Phase solubility studies

The phase solubility profile of DOM/βCD system is shown in Figure 1a. The profile can be classified as A_L type in distilled water and mannitol solution (1%).[24] The intrinsic solubility of DOM in water was found to be very low (0.0114 mM/ml). A linear increase in DOM solubility with increasing βCD suggests occurrence of soluble complexes with 1:1 stoichiometry. The slope of 0.00218 and 0.00211 also supports the formation of 1:1 complex between DOM and βCD in water and mannitol solution [Table 1]. The mannitol solution did not affect the solubilizing efficiency (SE) of DOM/βCD system remarkably. The value of $K_{c^{1:1}}$ for DOM/βCD system in water and mannitol solution was found to be 190.93/M and 146.53/M [Table 1]. Jun *et al*. have proposed that appearance of stability constant of drug/CD system in the range of 50-2000/M indicates the formation of a stable complex. As the values of the stability constants for

Table 1: Stability constants ($K_{c,1:1}$), solubilizing efficiency and Gibbs free energy (ΔG) of DOM/βCD and DOM/βCD/CA systems

DOM/βCD systems	Distilled water				1% mannitol			
	Slope	$K_{c,1:1}$	SE	ΔG (kJ/mol)	Slope	$K_{c,1:1}$	SE	ΔG (kJ/mol)
DOM/βCD	0.00218	190.93	0.00219	−13.53	0.00211	146.53	0.00211	−12.85
DOM/βCD/CA	-	-	0.02802	-	-	-	0.06409	-

SE: Solubilizing efficiency, DOM: Domperidone, βCD: β-cyclodextrin, CA: Citric acid

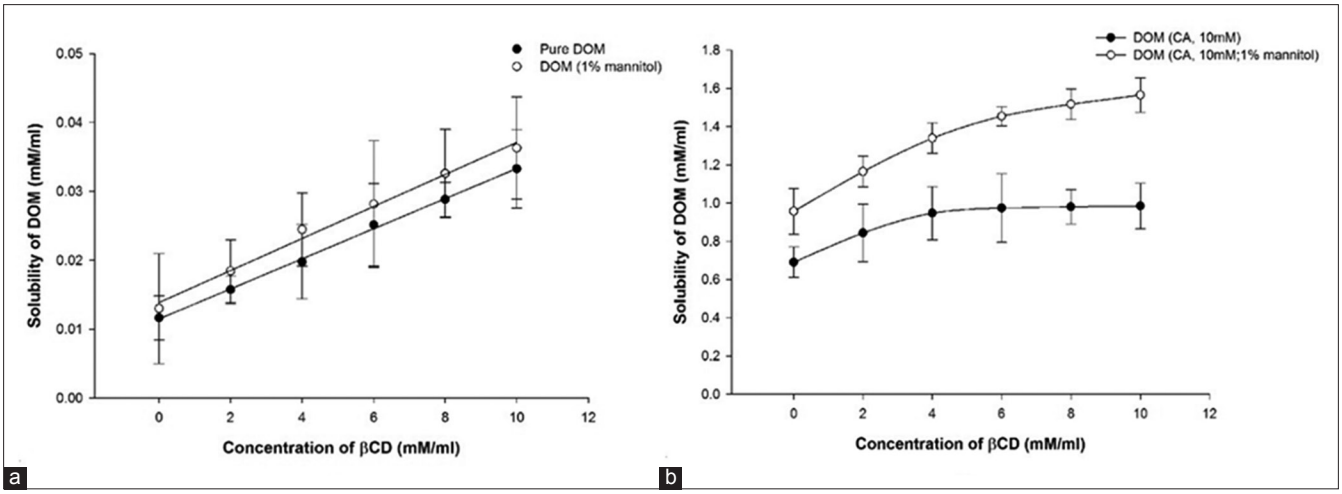

Figure 1: Phase solubility profile of domperidone/β-cyclodextrin system in distilled water (a) and citric acid solution (10 mM) (b)

DOM/βCD system lie in the same range, it indicates a stable association between DOM and βCD in water and mannitol solution.[31] The negative ΔG values indicated spontaneity of the complexation process with βCD.[32] A reduction in the stability constant of DOM/βCD system was observed in mannitol solution which may be due to competition of mannitol with DOM to form hydrogen bonds with βCD.

The phase solubility profile of DOM/βCD/CA in water and mannitol solution showed negative deviation from linearity (A_N type) which can be due to the change in solute-solvent interaction [Figure 1b]. The value of $K_{c,1:1}$ cannot be calculated from this solubility profile.[16] The SE of DOM/βCD/CA system was found to be increased by 13-fold as compared to DOM/βCD system in water whereas it was increased by 30-fold in mannitol solution [Table 1]. This clearly indicates a synergistic effect of CA and mannitol on solubility of DOM in the presence of βCD. According to Fenyvesi *et al*., hydroxy acids improve the solubilization and complexation of poorly basic drugs by forming hydrogen bonds with the hydroxyl groups of CDs. An occurrence of A_N type profile in case of DOM/βCD and DOM/βCD/CA systems indicates reduction in stability of the drug/CD complex.[33] This may be due to the ionization of DOM in the presence of CA. The ionized DOM has less affinity for the hydrophobic cavity of βCD.[34] However, more inclined nature of phase solubility profile of DOM/βCD/CA system in mannitol solution indicates that the presence of mannitol improves the solubility as well as the stability of the DOM/βCD/CA system. This may be due to the formation of co-complex, which is explained under characterization of inclusion complexes.

Drug content estimation

The total drug content of the physical mixtures and the kneaded complexes is shown in Table 2. The percentage drug content was found to be above 90% indicating minimum loss of product due to physical mixing and kneading method. The low values of standard deviation in the drug content indicated uniform drug distribution in physical mixtures and complexes.

Saturation solubility studies

The saturation solubility data for pure DOM, physical mixtures, and kneaded complexes are shown in Table 3. The physical mixtures and kneaded complexes showed significant enhancement ($P < 0.05$) in solubility of DOM. The QIC with equimolar ratio showed higher solubility than any other system. It was found that increase in the concentration of mannitol in quaternary systems caused marked improvement in the solubility of DOM.

As previously reported by Redenti *et al*., addition of hydroxy acid to a drug/CD system reduces the stability; however, it increases the overall solubility of the drug by combined approach of CD complexation and ionization of drug due to the acidic environment created by hydroxy acid.[15] In case

Table 2: Drug content of physical mixtures and kneaded complexes

System	Molar ratio of components	Drug content (μg/ml) (mean±SD)*
DOM/βCD (PM)	1:1	9.112±0.14
DOM/βCD/CA (PM)	1:1:1	9.601±0.31
DOM/βCD/CA/mannitol (PM)	1:1:1:0.25	9.105±0.19
DOM/βCD/CA/mannitol (PM)	1:1:1:0.5	9.564±0.48
DOM/βCD/CA/mannitol (PM)	1:1:1:1	9.726±0.54
DOM/βCD (KC)	1:1	9.082±0.36
DOM/βCD/CA (KC)	1:1:1	9.386±0.25
DOM/βCD/CA/mannitol (KC)	1:1:1:0.25	9.198±0.25
DOM/βCD/CA/mannitol (KC)	1:1:1:0.5	9.832±0.14
DOM/βCD/CA/mannitol (KC)	1:1:1:1	9.456±0.18

*Mean of three experiments. SD: Standard deviation, PM: Physical mixture, KC: Kneaded complex

Table 3: Solubility data of pure DOM, physical mixtures, and kneaded complexes

System	Molar ratio of components	Solubility in water at 25°C (μg/ml) (mean±SD)*	SEM
DOM	-	5.85±1.08	0.62
DOM/βCD (PM)	1:1	22.42±1.37	0.79
DOM/βCD/CA (PM)	1:1:1	48.58±1.18	0.68
DOM/βCD/CA/mannitol (PM)	1:1:1:0.25	78.11±0.81	0.47
DOM/βCD/CA/mannitol (PM)	1:1:1:0.5	104.24±1.12	0.65
DOM/βCD/CA/mannitol (PM)	1:1:1:1	132.63±0.92	0.53
DOM/βCD (KC)	1:1	56.37±1.27	0.73
DOM/βCD/CA (KC)	1:1:1	617.30±1.15	0.66
DOM/βCD/CA/mannitol (KC)	1:1:1:0.25	748.83±1.25	0.72
DOM/βCD/CA/mannitol (KC)	1:1:1:0.5	781.57±0.74	0.43
DOM/βCD/CA/mannitol (KC)	1:1:1:1	819.2±1.24	0.72

*Mean of three experiments. SD: Standard deviation, SEM: Standard error of mean, PM: Physical mixture, KC: Kneaded complex, DOM: Domperidone, βCD: β-cyclodextrin, CA: Citric acid

of ternary systems, CA improves the solubility of DOM by similar approach but the stability of the complex is found to be reduced.[34] Mannitol, being hydrotropic in nature, is capable of creating hydrophilic environment around DOM/βCD/CA system. In addition, it may form hydrogen bonds with the drug, βCD, and CA which may improve the stability of the system and further enhance the solubility of DOM to a greater extent. The involvement of mannitol in improving the stability of βCD in the presence of CA can be confirmed from the solid-state characterization studies.

Dissolution studies

DOM, being a weakly basic drug, exhibits better solubility at gastric pH (acidic) due to ionization, but at intestinal pH (alkaline), its solubility is significantly reduced.[9] The physical mixtures and the kneaded complexes were subjected to dissolution studies in 0.1 N HCl and phosphate buffer (pH 6.8) to study the effect of gastric and intestinal pH on the release of DOM. The dissolution profile of pure DOM, physical mixtures, and the kneaded complexes in the respective dissolution mediums is shown in Figures 2 and 3.

The physical mixtures and kneaded complexes showed significantly higher dissolution rate ($P < 0.001$) than the pure DOM in both mediums. On comparing the release profiles of physical mixtures with the kneaded complexes, it was found that physical mixtures showed slower dissolution than that of the kneaded complexes. The quaternary systems exhibited faster dissolution of DOM than that of the ternary and binary systems. In 0.1 N HCl, increase in the concentration of mannitol in the quaternary systems led to increase in the dissolution rate of DOM; however, this increase was found to be statistically insignificant ($P > 0.05$) which may be due to ionization of free as well as complexed drug. Within 5 min, QICs containing 0.25, 0.5, and 1 molar quantity of mannitol released 87.3%, 91.3%, and 98.5% of DOM in 0.1 N HCl, respectively, which was 3-4 times higher than the pure DOM. In phosphate buffer, the amount of DOM released within 5 min by the QICs was 72.8%, 79.0%, and 90.1% which was less as compared to the amount released in 0.1 N HCl. The reduction in dissolution of DOM at pH 6.8 can be attributed to poor solubility of DOM as mentioned before. However, QICs increased the dissolution of DOM by 23-28 times within 5 min in buffer.

The values of the dissolution parameters such as MDT and %DE calculated on the basis of dissolution profiles of physical mixtures and kneaded complexes in 0.1 N HCl and phosphate buffer are shown in Table 4. QIC containing equimolar amount of components showed highest %DE of 92.38% and 90.12% in 0.1 N HCl and phosphate buffer, respectively. As compared to other systems, the MDT of ternary complex (1.92 min)

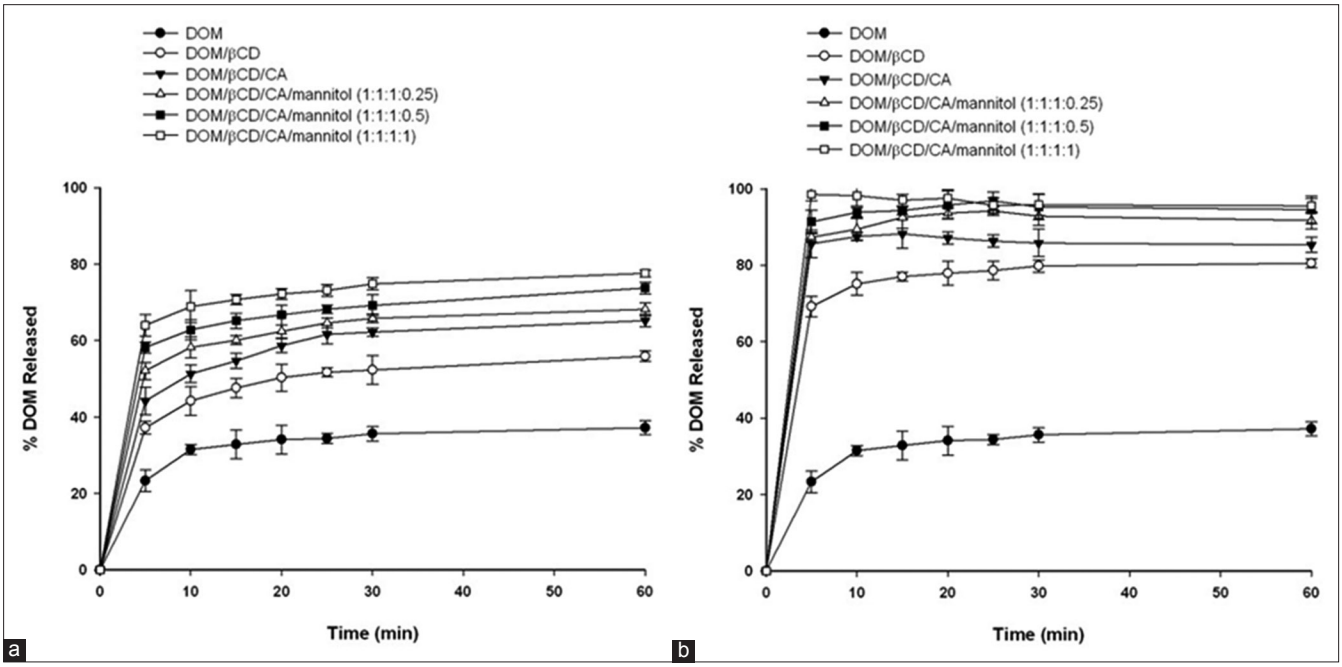

Figure 2: Dissolution profile of pure domperidone, physical mixtures (a), and the kneaded complexes (b) in 0.1 N HCl

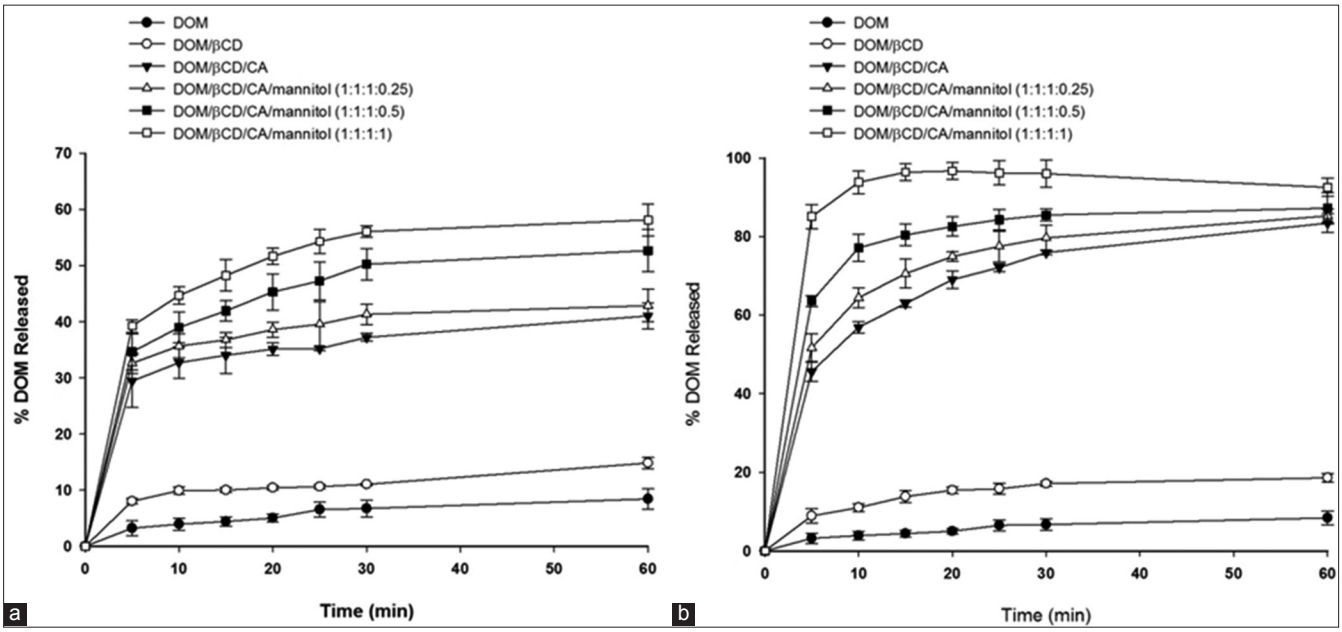

Figure 3: Dissolution profile of pure domperidone, physical mixtures (a), and the kneaded complexes (b) in phosphate buffer (pH 6.8)

and equimolar QIC (1.96 min) in 0.1 N HCl was found to be lowest, but their values were close to each other. The dissolution profile of ternary complex showed increase in the amount of DOM dissolved till 15 min (88.2%) after which a decline was observed indicating that DOM cannot be dissolved further due to poor solubility of noncomplexed DOM. On the other hand, equimolar QIC released 98.5% of DOM within 5 min followed by decline. Such difference in the dissolution pattern of ternary complex and QIC may be responsible for similar MDT of both systems. In phosphate buffer, the MDT of equimolar QIC was found to be lowest (1.54 min). It was found that increase in the amount of mannitol led to improvement in %DE at pH 1.2 and pH 6.8. MDT was found to be inversely proportional to the mannitol concentration in buffer; however, it showed anomalous behavior in 0.1 N HCl which may be due to ionization of DOM.

Characterization of inclusion complexes

The physical mixtures and kneaded complexes, both containing equimolar ratio of respective components, were

Table 4: Dissolution parameters of physical mixtures and kneaded complexes

System	Molar ratio	Dissolution medium			
		HCl (0.1 N)		Phosphate buffer (pH 6.8)	
		MDT (min)	%DE	MDT (min)	%DE
DOM/βCD (PM)	1:1	7.97	48.48	15.47	10.98
DOM/βCD/CA (PM)	1:1:1	7.56	56.98	8.83	34.97
DOM/βCD/CA/mannitol (PM)	1:1:1:0.25	6.31	61.03	6.77	38.01
DOM/βCD/CA/mannitol (PM)	1:1:1:0.5	6.81	65.38	8.56	45.15
DOM/βCD/CA/mannitol (PM)	1:1:1:1	5.65	70.29	7.65	50.70
DOM/βCD (KC)	1:1	4.19	74.88	11.47	15.05
DOM/βCD/CA (KC)	1:1:1	1.92	82.58	10.70	68.53
DOM/βCD/CA/mannitol (KC)	1:1:1:0.25	2.37	88.08	8.77	72.78
DOM/βCD/CA/mannitol (KC)	1:1:1:0.5	2.42	90.72	5.62	79.05
DOM/βCD/CA/mannitol (KC)	1:1:1:1	1.96	92.38	1.54	90.12

PM: Physical mixture, KC: Kneaded complex, MDT: Mean dissolution time, %DE: Percentage dissolution efficiency, DOM: Domperidone, βCD: β-cyclodextrin, CA: Citric acid

subjected to solid-state characterization studies along with pure DOM, βCD, CA, and mannitol.

ATR-FTIR spectroscopy

Infrared spectra of pure DOM, βCD, CA, mannitol, physical mixtures, and kneaded complexes are shown in Figure 4. The FTIR spectrum of DOM shows principle absorption peaks at 3118/cm and 3101/cm (N-H stretching of lactam), 3020/cm (aromatic C-H stretching), 2943/cm (asymmetric C-H streching), 2819/cm (symmetric C-H stretching), 1685/cm (C=O stretching), 1622/cm (aromatic C=C stretching), 1373/cm (C-H deformation), 1269/cm (C-N stretching), and 833/cm (C-Cl stretching). The IR spectrum of βCD shows prominent peaks at 3309/cm (O-H stretching), 2926/cm (C-H stretching), 1643/cm (H-O-H bending), 1151/cm (C-O stretching), and 1022/cm (C-O-C stretching). The spectrum of CA shows broad peak due to OH stretch at 3302/cm and sharp peak due to hydrogen bonded C=O stretch of carboxylic group at 1693/cm whereas spectrum of mannitol shows peaks at 3282/cm (O-H stretching), 2906/cm (C-H stretching), 1415/cm (C-H bending), and 1016/cm (C-O stretching).

The IR spectra of the physical mixtures show overlapping of the characteristic peaks of their respective components. In the spectrum of binary complex, the intensities of N-H and C=O stretching peaks of DOM showed more decrease as compared to the respective physical mixture. On other side, peak of DOM resulting due to aromatic C=C stretching disappeared. A slight shift was observed in the C=O stretching peak of DOM and C-O stretching peak of βCD which may be due to weak intermolecular interaction. It has been reported previously that changes in the FTIR spectra such as shift of characteristic bands, disappearance or reduction in intensity, and appearance of new bands indicate drug–CD interactions

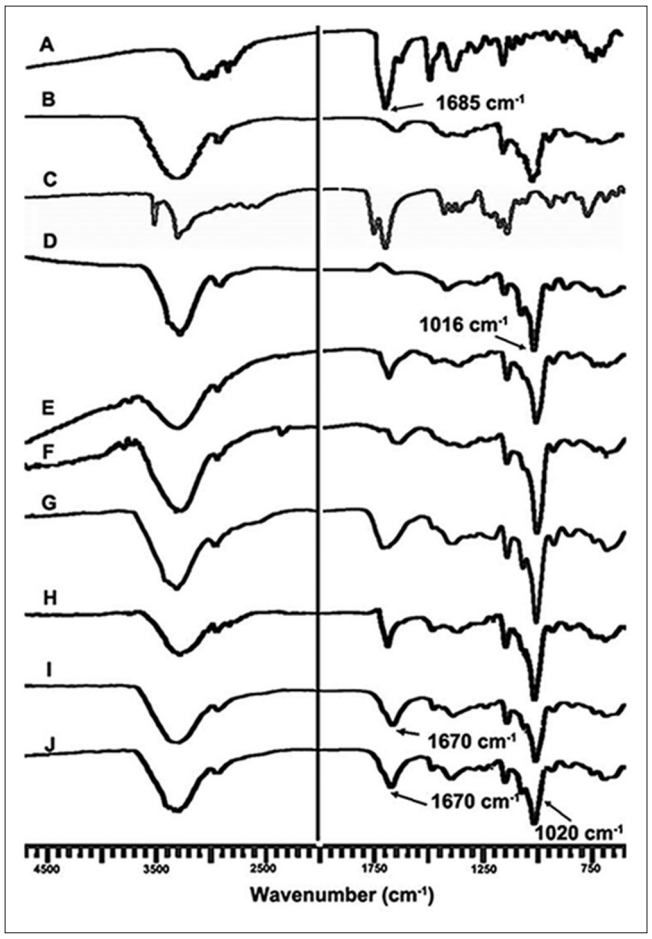

Figure 4: Infrared spectra of pure domperidone (DOM) (A), β-cyclodextrin (βCD) (B), citric acid (CA) (C), mannitol (D), physical mixtures of DOM/βCD (E), DOM/βCD/CA (F), DOM/βCD/CA/mannitol (G) and kneaded complexes of DOM/βCD (H), DOM/βCD/CA (I), and DOM/βCD/CA/mannitol (J)

or amorphization of product.[35] Overall, the spectrum of binary complex reveals the inclusion of benzimidazolone

group within the hydrophobic cavity of βCD. In case of ternary complex, a shift in the C=O stretching peaks of DOM was observed from 1685 to 1670/cm whereas the intensities of O-H stretching band of βCD and C-O stretching peak of CA were found to be increased. This suggests interaction between DOM, βCD, and CA.

In the spectrum of QIC, C-O stretching peak of mannitol at 1016/cm shifted to 1020/cm along with increase in intensities of O-H stretching band and C-O stretching peak which indicate interaction of mannitol with the other components of QIC which might involve weak forces such as van der Waals' interaction and hydrogen bonding. This indicates that mannitol might be involved in formation of a co-complex with DOM/βCD/CA molecules as reported previously by Ribeiro *et al*. in case of polymers.[36]

DSC

DSC thermograms of pure DOM, βCD, CA, mannitol, and kneaded complexes are illustrated in Figure 5. The thermogram of pure DOM displayed two endothermic peaks at 249°C and 397°C corresponding to the melting and decomposition of DOM. βCD showed a broad peak at 75-100°C which can be attributed to desolvation of water molecules present in βCD cavity and a relatively sharp peak at 337°C corresponding to its melting point. CA and mannitol exhibited sharp melting endotherms at 159°C and 170°C, respectively, followed by their decomposition above 200°C and 300°C.

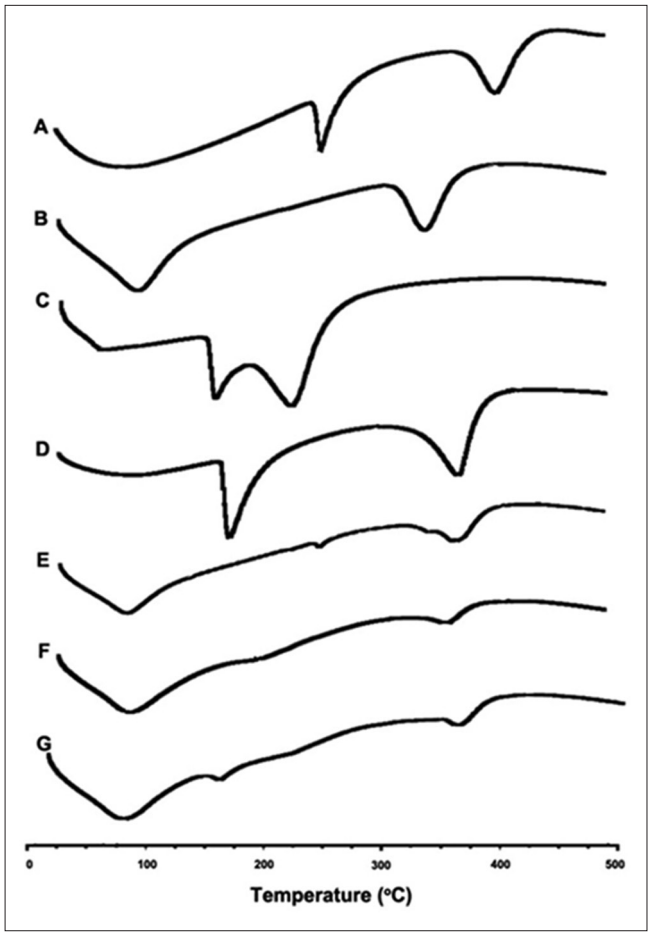

Figure 5: Differential scanning calorimetry thermograms of domperidone (DOM) (A), β-cyclodextrin (βCD) (B), citric acid (CA) (C), mannitol (D), and kneaded complexes of DOM/βCD (E), DOM/βCD/CA (F), and DOM/βCD/CA/mannitol (G)

It has been found that incorporation of the guest molecules into CD cavity leads to shifting or disappearance of their melting, boiling, and sublimation points.[37] For DOM/βCD kneaded complex, a prominent decrease in the endothermic peak of DOM was observed which suggests formation of inclusion complex between DOM and βCD. The thermogram of QIC clearly shows a shift in the endothermic peak of mannitol from 170°C to 164°C along with its decrease which suggests interaction of mannitol with other components of QIC as mentioned above. In the thermograms of ternary complex and QIC, the endothermic peak of DOM completely disappeared which may be due to increase in the complexation efficiency of βCD and molecular dispersion of DOM. This indicates that CA and mannitol play an important role in enhancing solubility of DOM by improving the SE of βCD along with ionization and molecular dispersion of DOM in acidic and hydrotropic environment.

XRD

XRD analysis was carried out to confirm reduction in crystallinity. Figure 6 depicts the diffraction patterns of DOM, βCD, CA, mannitol, and kneaded complexes. The spectrum of pure DOM exhibited sharp peaks at 13.71°, 22.15°, and 26.17° indicating its crystalline nature [Table 5]. The diffractograms revealed that βCD, CA, and mannitol were more crystalline than DOM as their peaks were more intense. There was a marked difference in the diffractograms of kneaded complexes and the individual components. A reduction in the diffraction peak intensities of DOM was observed for the kneaded complexes which indicate the amorphization of DOM [Table 5]. It was found that ternary complex and QIC showed rise in the peak intensities at certain angles than the binary complex. The increase in the peak intensities of inclusion complexes can be attributed to the formation of new solid crystalline phase upon complexation. Thus, the results of XRD analysis corresponding to the kneaded complexes indicated the presence of crystallinity; however, it did not affect the solubility and dissolution rate of DOM from the kneaded complexes due to its amorphization as previously mentioned.

SEM

The SEM photomicrographs of DOM and kneaded complexes are shown in Figure 7. The photomicrograph of pure DOM makes it clear that DOM was present in the form of crystalline flakes. In photomicrograph of DOM/βCD kneaded complex,

Table 5: XRD data for characteristic peaks of DOM, βCD, CA, mannitol and kneaded complexes

2θ	DOM	βCD	CA	Mannitol	Binary complex	Ternary complex	QIC
12.39	-	5191	-	-	2340	2159	1863
13.71	920	-	-	-	466	337	354
18.38	-	6304	-	-	701	598	734
18.6	-	-	6268.52	-	855	855	1054
18.62	-	-	-	8665	878	931	1172
19.76	-	3010	-	-	928	469	692
20.96	-	-	-	4134	845	879	1507
22.15	846	-	-	-	775	803	726
23.21	-	-	-	7349	463	584	1018
26.17	749	-	-	-	667	553	471
26.28	-	-	4580	-	748	607	480

DOM: Domperidone, βCD: β-cyclodextrin, CA: Citric acid, XRD: X-ray diffraction, QIC: Quaternary inclusion complex

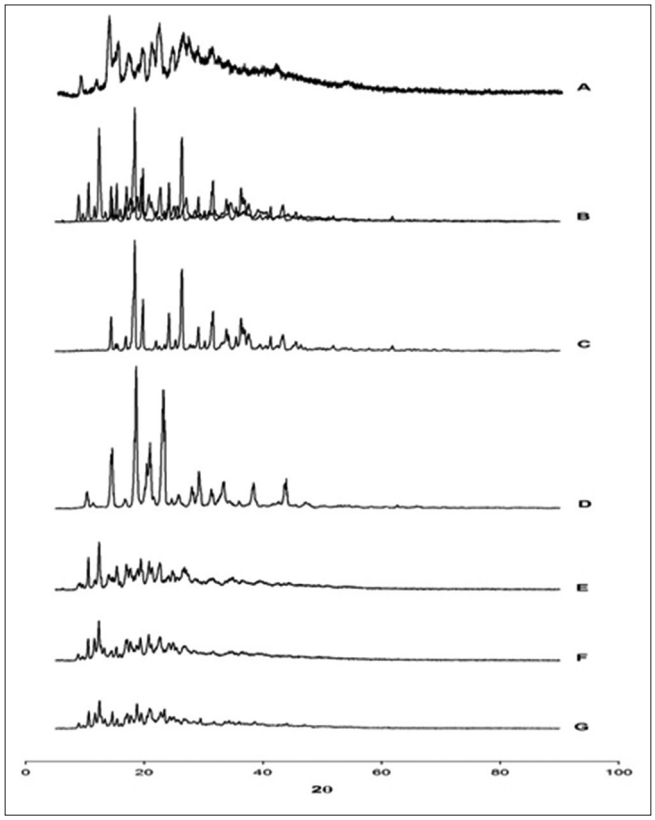

Figure 6: Diffractogram of domperidone (DOM) (A), β-cyclodextrin (βCD) (B), citric acid (CA) (C), mannitol (D), and kneaded complexes of DOM/βCD (E), DOM/βCD/CA (F), and DOM/βCD/CA/mannitol (G)

amorphous aggregates of small particles can be observed along with free DOM particles which might have led to poor solubility and dissolution rate of binary complex as compared to other complexes. On the other hand, complete disappearance of free DOM particles was observed in the photomicrograph of ternary complex and QIC. Besides, QIC exhibited rough surface which might have imparted more crystalline nature to QIC than the ternary complex as detected in XRD.

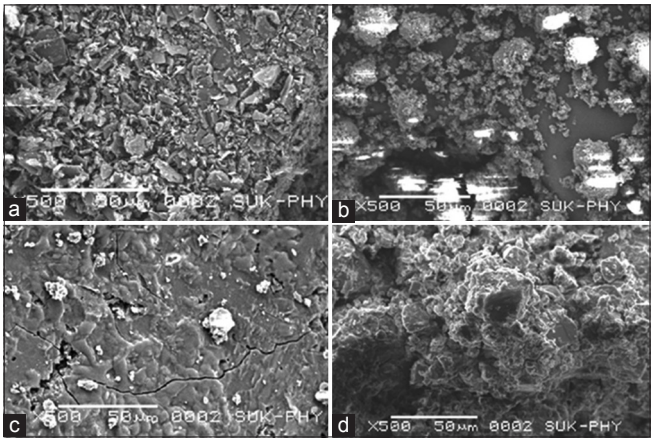

Figure 7: Scanning electron microscopy photomicrographs of domperidone (DOM) (a) and kneaded complexes of DOM/β-cyclodextrin (βCD) (b), DOM/βCD/citric acid (CA) (c), and DOM/βCD/CA/mannitol (d)

Thus, the results obtained from ATR-FTIR, DSC, XRD, and SEM analysis were in complete agreement with the results of our phase solubility, saturation solubility, and dissolution studies. It was found that enhancement in solubility of DOM obtained in case of ternary complexes and QICs was the result of various approaches such as inclusion interaction, generation of hydrophilic environment, reduction in crystallinity, and ionization of DOM. However, to some extent, the enhancement in solubility may also be attributed to self-assembly of CD molecules and adsorption of DOM on surface of βCD as reported by Ivanova *et al*.[38,39]

CONCLUSION

The results suggest that mannitol and CA extensively enhance the SE of βCD as compared to the DOM/βCD/CA system. Regardless of decreased affinity of DOM toward the hydrophobic cavity of βCD, an extreme improvement in

the solubility of DOM in QIC was observed. The equimolar QIC showed high %DE and low MDT as compared to any other system at alkaline pH. It was found that the increase in the amount of mannitol in the QICs lead to improvement in the dissolution parameters. The ATR-FTIR and DSC studies indicated non-covalent interaction between mannitol and other components of QIC. XRD and SEM analysis suggested the presence of crystallinity in the QIC; however, disappearance of characteristic peaks of DOM indicated its amorphization. Thus, the QICs prepared using mannitol along with CA can be a better alternative to the multicomponent systems composed of βCD, CA, and hydrophilic polymers. We also presume that the use of other derivatives of CD along with CA and mannitol may increase the solubility of poorly soluble weakly basic drug even further. The current approach can be beneficial in preparation of orodispersible or pH-independent extended release dosage form of such weakly basic drugs.

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