2017

Development and optimization of Dextromethorphan HBr-2-Hydroxy propyl ├ƒ-cyclodextrin inclusion complex based orally disintegrating tablets using response surface methodology

Saugat Adhikari
University of Toledo

A Thesis

entitled

Development and Optimization of Dextromethorphan HBr-2-Hydroxy Propyl ß-Cyclodextrin Inclusion Complex Based Orally Disintegrating Tablets Using Response Surface Methodology

by

Saugat Adhikari

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the

Master of Science Degree in

Pharmaceutical Sciences, Industrial Pharmacy

_____

Kenneth S. Alexander, PhD, Committee Chair

_____

Sai Hanuman Sagar Boddu, PhD, Committee Member

_____

Jerry Nesamony, PhD, Committee Member

_____

Amanda Bryant-Friedrich, PhD, Dean
College of Graduate Studies

The University of Toledo

August 2016

An Abstract of

Development and Optimization of Dextromethorphan HBr-2-Hydroxy Propyl ß-Cyclodextrin Inclusion Complex Based Orally Disintegrating Tablets Using Response Surface Methodology

by

Saugat Adhikari

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the Master of Science Degree in Pharmaceutical Sciences, Industrial Pharmacy

The University of Toledo

August 2016

The focus of this present investigation was to access the utility of various characterization techniques in the evaluation of Dextromethorphan HBr (DXM HBr) inclusion complex with 2-Hydroxy propyl ß-cyclodextrin (2-HPßCD). This techniques confirms the formation of the inclusion complex and explores the mode of complexation between DXM HBr and 2-HPßCD. It also predicts the ability of 2-HPßCD to mask the bitter taste of DXM HBr and explain its taste masking mechanism. In aqueous solution, the inclusion complex was studied utilizing the phase solubility method. The solubility of DXM HBr increased as a function of 2-HPßCD concentration. The solubility profile was classified as A_L type: indicating the formation of a 1:1 stoichiometric inclusion complex. In solid state, the inclusion complex was prepared using lyophilization (freeze drying technique) and characterized by Differential Scanning Calorimetry (DSC), Fourier Transform Infrared (FT-IR), Scanning Electron Microscopy (SEM), powder X-ray Diffraction (pXRD), proton nuclear magnetic resonance (^{1}HNMR) spectroscopy and 2D-NMR

rotating Over Hauser effect spectroscopy (ROESY). FT-IR showed no interaction between DXM HBr and 2-HPßCD and confirmed the formation of the complex. DSC and SEM studies further confirmed the inclusion complex formation. pXRD analysis indicated that the crystallinity of the inclusion complex reduced significantly. NMR spectroscopy elucidated the mode of complex formation. The subsequent incorporation of the inclusion complex into orally disintegrating tablets (ODTs) was done to develop the formulation. This results in patient adherence and convenience and enhances the dissolution rate by rapid absorption of drug through oral mucosa. Response surface methodology with central composite design was employed in the optimization of the formulation factors, such as concentration of croscarmellose sodium (CCS) and microcrystalline cellulose (MCC), to obtain ODTs within the range of 3.5 to 5.5 kp hardness, 6.3 to 45 second disintegration time and 1.2 to 6.06 minutes mean dissolution time (MDT). The results indicated selected factors which have a strong influence on properties of the ODTs. The optimum concentration of CSS and MCC predicted by the model was 5.168 mg (2.5%) and 81.814 mg (40%), respectively for preparing a DXM HBr-2-HPßCD inclusion complex based ODT with a hardness of 4.5 kp, disintegration time of 10 seconds and MDT of 1.341 minutes. Thus, this approach exhibited the ability of masking the bitter taste of DXM HBr when complexed with 2-HPßCD, which resulted in ODTs formulations with improved patient adherence and acceptability.

Acknowledgements

This project is not the result of one individual's effort, but is the product of collective experience of all those who have shared their views for this work. I would like to express my deep sense of gratitude to my major advisor Dr. Kenneth S. Alexander for his valuable guidance, suggestions, thought-provoking discussions and constructive criticism which always inspired me to work diligently.

I would like to thank my thesis committee members: Dr. Sai Hanuman Sagar Boddu and Dr. Jerry Nesamony for their advice, encouragement and support over the last two years. I am sincerely thankful to Dr. Caren Steinmiller for serving as a graduate faculty representative for my thesis defense. I would also like to thank Dr. Pannee Burckel for helping me with the scanning electron microscopy and X-ray diffraction studies. I thank Roquette America Inc. for providing me with samples of 2-Hydroxy Propyl ß-cyclodextrin. I also thank JRS Pharma for providing me with the tablet excipients samples. My sincere thanks to the Department of Pharmacy Practice, The University of Toledo for providing me with financial support throughout the tenure of my study. Over these years in Toledo, I have come across many individuals for whom I have great regard. A special thank you to my friends and colleagues who have stood by me in all my efforts for successfully completing this work. My deepest gratitude goes to my family for their unflagging love and support throughout my life. I would like to thank all of you in making this project a worthy endeavor.

Table of Contents

List of Tables

List of Figures

Chapter 1

Introduction

1.1. Pediatric Medications

Most children face difficulties in taking prescribed medications. Many active pharmaceutical ingredients (API's) are bitter in taste and unpleasant for children and adults as well. API's can be encapsulated to reduce the bitter taste and increase patient adherence in adults. However, it is still problematic for many children as they will not swallow encapsulated medications or pills easily [1, 2]. Even though children suffer from the same kind of diseases as adults and the same drug is used for the treatment of those diseases, pediatric formulations need to be revised and modified to achieve better safety and efficacy of the dosage form in children [3]. Formulations which are child unfriendly, provide a high risk of adverse consequences with alteration in medication regimen and suboptimal dosing [2]. Children may also fail to adhere to the medication regimen and may cause serious adverse effects. The absence of critical safety and efficacy information for pediatric formulations have significant risks to children [4].

1.1.1. Problems associated with Pediatric Medications

The formulation development of pediatric medications is an area with many challenges and problems for a research scientist. The bad taste of medications and pediatric formulation challenges have been a major problems associated with children's medicine [5].

Bitter taste of the API

One of the most important requirements that the pediatric formulation must meet is palatability. Bitter taste of the API's makes medications unpalatable [6]. Children will no doubt reject this unpalatable medicine. Many of the pharmaceutical API's by nature have very bitter taste. This bitter taste can be attributed to the sensory expression of its pharmacologic activity. An increase in bitterness will increase the extent that the medication will be rejected by the child [5].

Mechanism of the bitter taste

Food molecules interact with the saliva and bind to the taste receptor. Sensation of taste is due to the presence of taste receptors in the mouth [7].

The main sensory organ for gustation is the taste bud which is comprised of 50-100 epithelial cells and some of which are receptors. When the receptor proteins are expressed in the epithelial cells, they stimulate the receptor cells. Saliva also plays a significant role in the activation of the receptor cells and is responsible for the reduction of the extent of bitterness [2, 6]. The pathway of the gustatory signals through the brain is responsible to produce the taste signals. Taste signals can also be modulated along the

central gustatory pathway and not only in the periphery. The front of the tongue increases perceived bitterness as compared to the back of the tongue, probably due to prevention of the inhibition from the anterior lingual taste signals [7].

There are two different classes of taste receptor mechanisms, the G-protein coupled receptor (GPCR) is responsible for sweet, bitter and umami taste and the ion channels receptor is responsible for salt and sour taste [8]. If the activities and transduction intermediates of these receptors are thermally sensitive, temperature can also be used for modulating the taste of the medicine. The GPCR share transduction intermediates in taste receptor cells which releases the neurotransmitter. The T2R family of taste receptors consists of about 25 GPCR receptors for modulating the bitter taste. The T2R receptors mainly bind with different bitter tasting ligands and activate them. The temporary deactivation of the GPCR transduction signaling components can help in reduction of the bitterness of the medicine and increases its palatability [2, 7].

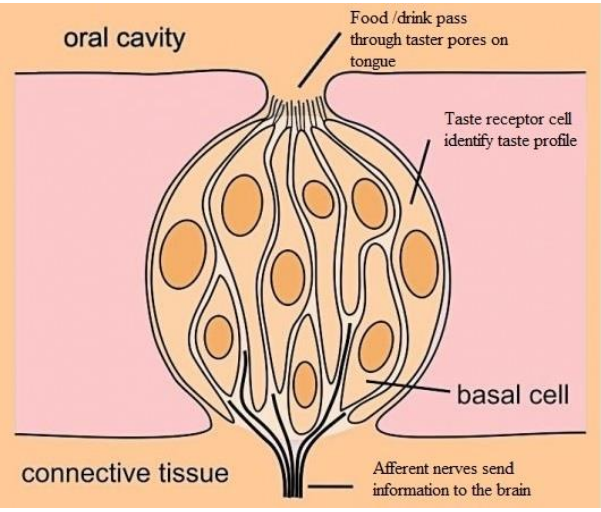


Figure 1.1: Mechanism of taste

Modified from https://en.wikipedia.org/wiki/Taste

The mechanism of the bitter taste is due to the signaling from the taste receptor [2]. However, with many bitter tasting compounds it is not possible to reduce the bitterness at the receptor level because the drug may stimulate multiple receptors which requires its own antagonist [7]. Several components of the bitter taste transduction signaling pathway are shared with those meditating sweet taste and it may create the problem to reduce the perceived bitterness. Hence, temporary non selective blocking of these bitter taste transduction signaling pathways can increase the palatability of the medicine [2, 5].

1.1.2. Formulation challenges of the pediatric dosage forms

Children are not just small humans, but they are small from a biological and pharmacological development perspective. Physiology (biology) of children, age, size and treatment requirements are the challenges in formulating safe and effective medications for them [5]. Inadequate drug formulations may cause problems in children's which are not observed in adults. These include a difficulty in swallowing conventional size tablets, excipient interactions, safety issues and patient adherence problems due to palatability [8]. The lack of attention regarding age appropriate medication therapy gives rise to ethics related problems where adult medicines are used off-label in children which carries additional risks [9].

Non-adherence to the medication is mostly due to the pain, discomfort and unnecessary burden on children during drug administration [10]. The matter of taste in pediatric oral formulations can cause patient non-adherence. The low tolerance of children for disagreeable taste influences the loss of medication from spillage or spitting [11].

The selection of preservatives, sweeteners, fillers and solvents is challenging as there have been some evidence that suggests excipients commonly used in adult medication may cause elevated toxicity and safety issues in children [9, 12].

Aspartame is used as a sweetener in beverages, food products and pharmaceutical preparations. It can mask the taste of the bitter drugs and helps in formulating pediatric medications with a pleasant taste. However, a number of adverse events have been reported associated with hyperactivity in children due to the aspartame consumption [13].

Poorly soluble drugs normally prepared as oral suspensions use various surfactants to aid the wetting and dissolution of the drugs. Surfactants such as docusate sodium and polysorbates produce a series of adverse events in children if their concentration level is not controlled in pediatric medications [14].

Buffering agents, anti-oxidants and preservatives are used for physical, chemical and microbiological stability. However, preservatives such as benzyl alcohol and sodium benzoate have produced fatal adverse events in pediatric populations [15, 16].

The crucial importance is to mask the unpleasant taste of formulations with sweeteners and flavors. Oral liquid flavors that usually mask the taste of drugs are complimented with colorants which may cause allergic reactions and hypersensitivity in children [3]. When this is not achievable, sophisticated formulation approaches such as encapsulation and complexation should be prepared which also increases technical challenges and lengthens the process thus making the formulation expensive [4].

Conventional oral solid dosage forms such as tablets are associated with the risk of choking and have limited dose flexibility [17]. Liquid formulations may possess the

problems of palatability and dose uniformity. In addition, stability (chemical, physical and microbiological) is another major problem with liquid formulations. Liquid formulations can be bulky and have difficulties during handling and shipping [3, 9].

Even with medications which have been approved and dosing regimens authorized for children, the availability of appropriate dosage forms is limited [11]. Cleary these formulation challenges focus the need that more research and development are required.

1.1.3. Bitter tasting pediatric drug : Dextromethorphan Hydrobromide

Many of the active pharmaceutical ingredients either during or immediately after oral absorption exhibit undesirable characteristics such as bitter taste. Acetaminophen, azithromycin, ampicillin, chlorpheniramine, dextromethorphan, diphenhydramine, ibuprofen, penicillin, pseudoephedrine, ranitidine, spironolactone and theophylline are among a few of the API's or medicinal agents that have characteristic bitter taste [18, 19].

Dextromethorphan (3-methoxy-N-methylmorphinan) is one of the most commonly used antitussive drug (cough suppressant) in children. Dextromethorphan has an opioid like structure. However, being the d-isomer it does not possesses the analgesic/addictive properties of opioids. Dextromethorphan was approved as a non-prescription cough medication in 1958 by the FDA [20]. In current scenarios, dextromethorphan can be found in more than 125 OTC cough and cold patented products. It is available as pills, gels, caps, lozenges, liquids and syrups but its availability as a solid dosage form is limited. It is given either alone or in combination with analgesics (acetaminophen), expectorants (guaifenesin) and/or antihistamines (brompheniramine, chlorpheniramine

and diphenhydramine). It's mainly active against the dry cough and is used in combination with expectorants to have significant effects for productive cough [21].

1.1.3.1. Physiochemical properties of dextromethorphan

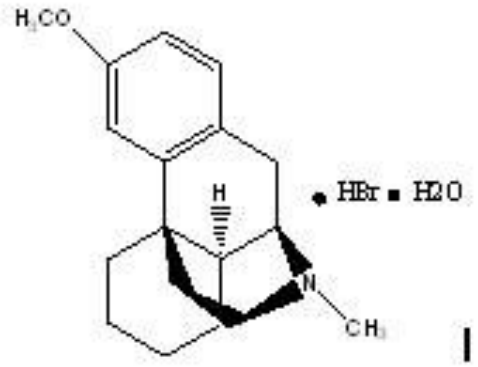


Figure 1.2: Structure of Dextromethorphan HBr

Chemical name: Dextromethorphan hydrobromide

Molecular formula: $C_{18}H_{25}NO.HBr.H_20$

Molecular weight: 370.3

CAS Registry: 6700-34-1 (Dextromethorphan Hydrobromide, monohydrate)

A white crystalline powder, with faint odor

Soluble in 65 parts of water (USP) and 1 in 10 parts alcohol; freely soluble in chloroform and practically insoluble in ether [20].

1.1.3.2. Pharmacology of dextromethorphan

After oral administration, dextromethorphan hydrobromide is rapidly absorbed from the GIT. The onset of action is between 15-30 minutes and the peak serum level is achieved within 2.5 hrs. It undergoes rapid first pass metabolism. The metabolism involves the oxidative enzyme cytochrome P4502D6/ CYP2D. Elimination half-life of the drug is about 2-4 hours in the majority of individuals but it may be 24-72 hours in slow metabolizers [20, 22].

1.1.3.3. Safety and dosage of dextromethorphan

The oral dose of dextromethorphan in adults is 10- 20 mg every four hours, 30 mg every 6-8 hours, with a maximum of 120 mg in 24 hours. Children from 6-12 years are given 5-15 mg every 4-8 hours to the maximum of 60 mg in 24 hours. Children from 2-6 years of age are given 2.5-5 mg every 4 hours, 7.5 mg every 6-8 hours to the maximum of 30 mg in 24 hours [21].

Clinical studies show that a single dose of 120mg/day may produce some adverse effects. Ingestion of 10 mg/kg or less is unlikely to produce toxicity in child. There is a greater potential for toxicity in children with long acting preparations [22].

1.1.3.4. The problem: Bitter taste of the dextromethorphan

Dextromethorphan in cough and cold syrups provides the main problem of bitterness which leads to patient non-adherence, especially in children. There are various oral

solid/liquid dosage forms which contain dextromethorphan as the active ingredient and are bitter in taste [23, 24]. Due to the presence of the amine functional group in dextromethorphan hydrobromide, the drug has an obnoxious taste. The amino functional group in its molecular structure is responsible for the characteristic bitter taste [24]. There have been some studies which show that the amine functional group can be blocked by the formation of a complex and results in reduction in the bitterness of the product. However, there are no detailed studies or data which show the blockage of the amine group during the formation of the complex.

1.1.3.5. The problem: Formulation challenges of dextromethorphan

Dextromethorphan hydrobromide is readily absorbed in the upper GIT and has short biological half-life. It belongs to BCS- class II drugs and exhibits low solubility and high permeability. Because of these reasons, the bioavailability of the drug is drastically low in conventional oral solid/liquid preparations [19]. In addition, most of the commercial formulation available for dextromethorphan are oral liquid formulations (syrups and suspensions) e.g. Deslym® [18, 19, 23]. These oral liquid dosage forms are bulky and the stability of these formulations can be key issues. Also, with the oral liquid formulations we face patient non-adherence and dosing problems, especially with pediatric formulations of dextromethorphan [18, 25].

1.2. Cyclodextrins as drug carrier

Designing advanced dosage forms requires suitable carrier materials to overcome the undesirable properties of drug molecules. Cyclodextrins (CDs) can alter the physical chemical and biological properties of the guest molecules through the preparation of inclusion complexes [25]. They are pharmaceutical excipients that can solubilize various poorly water soluble drugs by forming water soluble drug cyclodextrin complexes [26].

Chemisty

The α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin are widely used natural cyclodextrins, consisting of six, seven and eight D-glucopyranose residues, respectively, linked by α-1,4 glycosidic bonds into a macrocycle [27-29]. The structure is shown in *Figure 1.3*. X-ray investigation of cyclodextrin molecules revealed that due to the presence of primary and secondary hydroxyl groups as well as due to its hydrophopic cavity each cyclodextrin has its own ability to form inclusion complexes with specific guests, which depends on a proper fit of the guest molecule into the hydrophobic cyclodextrin cavity [25].

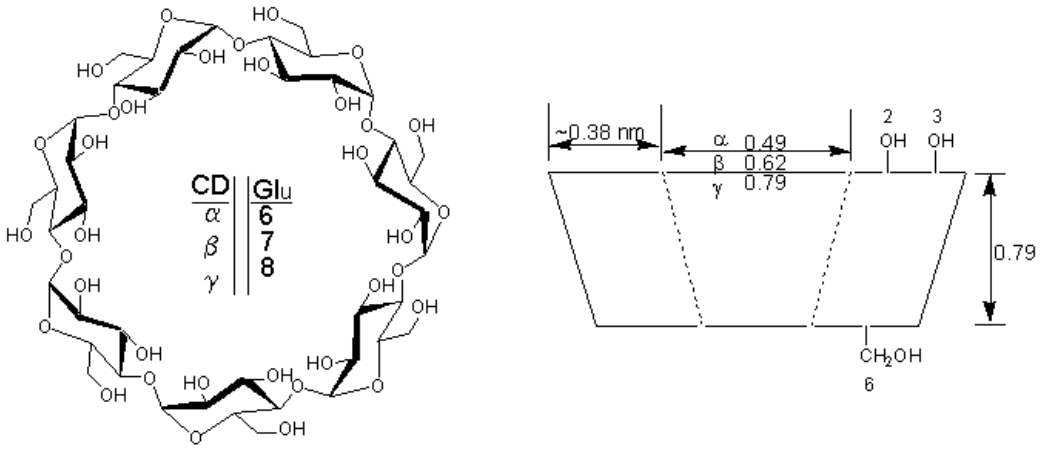


Figure 1.3: Structural features of cyclodextrins [27, 30]

The most common pharmaceutical application for cyclodextrins is to enhance solubilty, stability and bioavailability of drug molecules, and to mask the unpleasant taste of drugs [31]. However, natural cyclodextrins have relatively low solubility in water and organic solvents, thus restricting their use in pharmaceutical formulations [32, 33]. Recently, various types of cyclodextrin derivatives have been prepared to improve their physicochemical properties and inclusion capacity of natural cyclodextrins as novel drug carriers [34].

Physical Properties

The dimension of the cyclodextrin changes with the change in the number of glucose units. Because of the difference in internal cavity diameters, each cyclodextrin shows a different capability of inclusion complex formation with differently sized guest molecules [30, 35]. *Table 1.1* lists the dimensional sizes as well as important physicochemical characteristics of the different types of cyclodextrins.

Table 1.1: Physical properties of the cyclodextrins [25, 29, 35]

Sr. No	**Characteristics**	**α-cyclodextrin**	**β-cyclodextrin**	**γ-cyclodextrin**
1	Number of glucose unit	6	7	8
2	Molecular weight	972	1135	1297
3	Central cavity diameter (A^0)	4.7-5.3	6.0-6.5	7.5-8.3
4	Water solubility(g/100ml at RT)$^{\#}$	14.5	1.85	23.2
5	Optical rotation $(\alpha)_D^{25°C}$	150+0.5	162.5+0.5	177.4+0.5

6	Cavity diameter (A^0)	4.7-5.3	6.0-6.5	7.5-8.5
7	Height of torus (A^0)	7.9±0.9	7.9±0.9	7.9±0.9
8	Diameter of outer periphery (A^0)	14.6+0.4	15.4+0.4	17.5+0.4
9	Approximate volume of cavity (A^0)	174	262	427
10	Approximate volume of cavity in 1 mol-cyclodextrin(ml)	10	157	250
11	Melting point	275	280	275

[#] Aqueous solubility in grams per 100 ml of water at ambient temperature

1.2.1. β-cyclodextrin

The structure of β-cyclodextrin is shown in *Figure 1.3*. As a consequence of C_1 confirmation of α-D-glycopyranosyl residues and lack of free rotation around glycosidic bonds, the compounds are not cylindrical but somewhat cone-shaped [36]. The secondary hydroxyl groups (on the C-2 and C-3 atoms of the glucose units) are situated on one edge of the ring and all primary hydroxyls on other and this makes the cyclodextrin exterior hydrophilic [27]. It is shown in *Figure 1.4*.

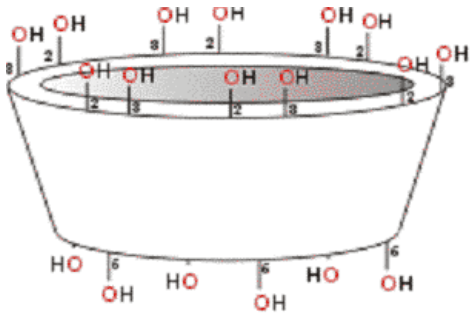


Figure 1.4: Location of secondary and primary hydroxyl groups in β-cyclodextrin [35]

The main properties of β-cyclodextrin include less irritation to GIT compared other types of cyclodextrins, less absorption in the upper GIT and metabolized by bacteria present in colon [37]. Currently, ß-cyclodextrin is the most common type of cyclodextrin in pharmaceutical formulation. β-cyclodextrin has low solubility in water compared to its derivatives. Many intermolecular hydrogen bonds exist between secondary hydroxyl groups. These intermolecular hydrogen bonds help in stabilizing the macrocycle of the cyclodextrin molecule and turn the β-cyclodextrin molecule into a rigid structure [34]. These intermolecular hydrogen bonds also prevent hydration of the cyclodextrin molecule, which may be the cause for the low solubility of β-cyclodextrin [30].

1.2.2. 2-Hydroxy Propyl ß-cyclodextrin

2- Hydroxyl propyl β-cyclodextrin (HP βCD) is a hydroxyalkyl derivative, an alternative to α, β and γ-cyclodextrin which has improved water solubility properties and reduced toxicity to humans [38]. Its structure is shown in *Figure 1.5*. The following combined characteristic features of HP βCD offers huge formulation opportunities [39, 40]:

a. HP βCD has high aqueous solubility and is infinitely soluble in water at room temperature.
b. The process of complexation is much facilitated compared with native cyclodextrins
c. The safe biological profile of HP βCD permits for wider use and administrative routes.

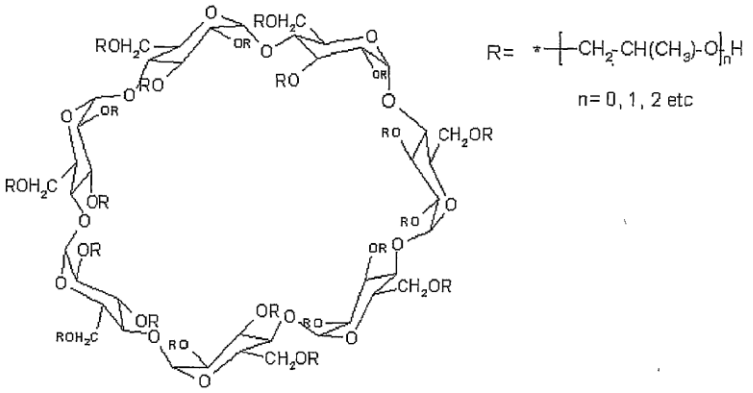


Figure 1.5: Structure of Hydroxyl propyl β-cyclodextrin [41]

HP βCD can be found in several marketed drug formulations with oral dosing of up to 8 g HP βCD /day and intravenous dosing of up to 16 g HP βCD /day (Itraconazole®) [35, 38]. HP βCD has been shown to be well tolerated in humans. The oral bioavailability of HP βCD in humans is between 0.5 to 3.3% with 50 to 65% of the oral dose excreted intact in the feces with the remainder mainly being metabolized by the intestinal microflora [37].

1.2.3. Cyclodextrin Inclusion Complexes: Requirements for complex formation

1.2.3.1. Geometric Compatibility

Cyclodextrins feature an ability to form inclusion complexes by trapping the various guest molecules into its cavity. The minimum requirement for this inclusion complex formation is that the guest molecule must fit entirely or at least partially, into the cyclodextrin cavity. Stable complexes will not be formed with guest molecules which are too small to be enclosed by the cyclodextrin molecules because they will slip out of the

cavity. Complex formation is also impossible with molecules which are too bulky to penetrate into the cyclodextrin cavity. However if certain groups or side chains of the bulky molecule can penetrate into the cyclodextrin cavity, complex formation remains possible [42]. Most frequently the host-to-guest ratio is 1:1 for molecular encapsulation. However 2:1, 1:2, 2:2 or even more complicated associations and higher equilibria can exist [33] .

1.2.3.2. Polarity and Charge

Hydrophobic molecules or residues have higher affinity than hydrophilic molecules for the cyclodextrin cavity in aqueous solution. Hydration of a hydrophobic guest is generally favored as compared to separate hydration of the components. This hydrophobic interaction is due to the intrinsic cohesion of the water molecules and not to the mutual attraction of the two components [43].

1.2.3.3. Binding forces of the Complexes

Cyclodextrin complexes are stabilized by various intermolecular forces such as [44]:

a. Van der waals interaction between the guest and host. The Van der waals forces here include both permanent induced-dipole-dipole interactions and London dispersion forces.

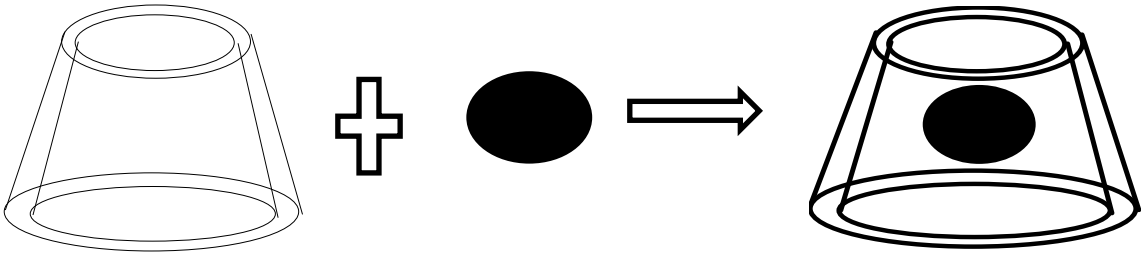
b. Hydrogen bonding between the guest and host.

c. Release of high-energy water molecules in complex formation. Inclusion complex formation replace these high enthalpy water molecules by guest compounds, resulting in a favorable enthalpy change.

d. Release of strain energy in the macromolecular rings of the cyclodextrin. It causes the change in high energy conformation of the cyclodextrin-water complex to the lower energy conformation of the cyclodextrin-guest complex.

1.2.4. Cyclodextrin complexes and Phase Solubility

Cyclodextrins can form inclusion complexes with the guest molecule in aqueous solution by engulfing the lipophilic moiety of the drug molecule into the hydrophobic central cavity of cyclodextrin [31]. This is seen in *Figure 1.6*. In aqueous solution, drug molecules within the cyclodextrin cavity are in dynamic equilibrium with free drug molecules and there are no covalent bonds formed or broken during the formation of the complex. This is a diffusion controlled mechanism and drug cyclodextrin complexes are continuously being formed and dissociated [36].



Cyclodextrin Cavity Drug Molecule 1:1 Drug-CD complex

Figure 1.6: Schematic of 1:1 drug-cyclodextrin complex formation

The affinity for the drug to cyclodextrins in the formation of the complex is given by the stability constant (equilibrium constant) K. The K-value is determined by titrating changes in the physicochemical properties of the drug molecule within the cyclodextrin and analyzing the concentration dependencies [45]. Physicochemical properties of guest molecules such as aqueous solubility, stability, molar absorptivity, NMR chemical shifts, pka values and HPLC retention times can be studied [35].

The most commonly used method to study the inclusion complexation is the phase solubility method described by Higuchi and Connors. This method studies the effect of solubilizer CD (ligand) on drug molecule being solubilized (substrate). Phase solubility diagrams are generally categorized as two types [45]:

a. Type A diagrams
b. Type B diagrams

Type A diagrams:

Type A curves indicate the formation of the soluble inclusion complexes. They are further sub-divided into;

A_L type: linear increase in drug solubility as the function of CD concentration

A_P type: positively deviating isotherms

A_N type: negatively deviating isotherms [45]

This is shown in *Figure 1.7a*. Chemically modified cyclodextrins, such as HPßCD, which usually have higher solubility, produce the soluble complexes and give Type-A diagrams [28, 46].

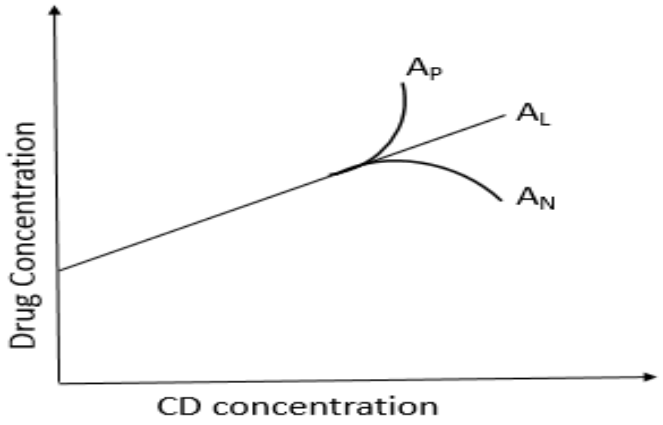


Figure 1.7a: Type-A diagrams

Type B diagrams:

Type B curves suggest the formation of inclusion complexes with poor solubility. The B_S-type diagram represents the complexes with limited solubility and B_I-curves indicate the formation of insoluble complexes. This is shown in *Figure 1.7b*. Native ß- CD, due to its limited solubility, gives rise to B-type diagrams [29, 32].

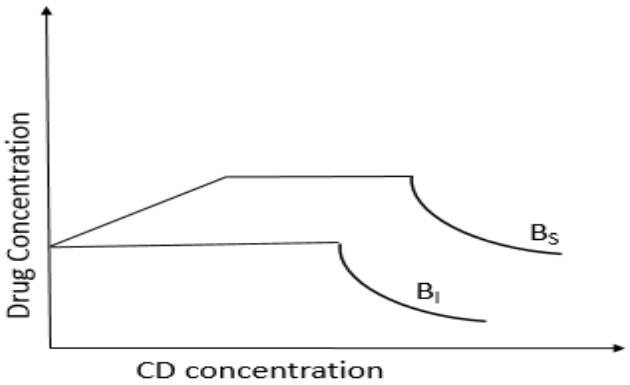


Figure 1.7b: Type-B diagrams

In the case of 1:1 complexes, the stability constant (equilibrium constant) $K_{1:1}$ can be expressed as the slope of the linear portion of the curve.

It is given by [45];

$$K_{1:1} = \frac{slope}{S_0\,(1-slope)} \quad \text{.....Eqn. (1.1)}$$

where S_0 is the intrinsic solubility of the drug molecule.

1.2.5. Mechanism of Drug Release from the Inclusion Complex

Equilibrium binding of the drug and CD complexes (1:1 complex) is represented by [28, 29];

$$Drug + CD \leftrightarrow Drug{:}\,CD\ complex \quad \text{.....Eqn. (1.2)}$$

For 1:1 inclusion complex, the magnitude of binding constant $K_{1:1}$ is given by [28];

$$K_{1:1} = \frac{[drug]_{complex}}{[drug]_{free}[cyclodextrin]_{free}} \quad \text{.....Eqn. (1.3)}$$

where $drug_{complex}$ represents concentration of the drug in complex;

$[drug]_{free}$ represents free drug concentration; and

$[cyclodextrin]_{free}$ represents the concentration of free cyclodextrin.

Intrinsic drug solubility is given by [28, 46];

$$[drug]_{total} = [drug]_{intrinsic} + \frac{K_{1:1}[drug]_{intrinsic}[cyclodextrin]_{total}}{K_{1:1}[drug]_{intrinsic} + 1} \quad \text{..Eqn. (1.4)}$$

where $[drug]_{total}$ represents total drug solubility;

$[drug]_{intrinsic}$ represents intrinsic solubility;

$[cyclodextrin]_{total}$ represents total molar concentration of cyclodextrin in solution.

Depending upon the phase solubility behavior of the system, the ratio of free to complexed drug upon the dilution of drug-CD complexes can be determined. When the relationship between the drug solubility and cyclodextrin concentration is linear, 1:1 inclusion complex dilution will not result in the precipitation of the drug. However, precipitation may occur if the relationship between drug solubility and CD concentration is non-linear [45]. Hence, dilution effects the release of the drug from the inclusion complex.

Hence**,** the drug release from the cyclodextrin inclusion complex is based on the relationship between intrinsic drug solubility, the magnitude of the binding constant (stability constant) for the inclusion complex and dilution effects.

1.2.6. Preparation of Inclusion complexes using a Freeze Drying Technique (Lyophilization)

This research implemented a solvent evaporation method using a freeze drying technique for the formulation of the complex. The drug and the host molecule are dissolved in a common solvent and the solvent is removed by freeze drying. The solvent system from the solution is eliminated through primary freezing and subsequent drying of the solution containing both drug and CD at the reduced pressure. Lyophilization techniques offer the

advantage of getting porous amorphous powder with a high degree of interaction between drug and CD. This method is useful for the complexation of thermolabile drugs. The only limitation of this process is the poor flow properties of the powdered product as well as it being a time consuming process [47, 48].

Other commonly used methods for the preparation of a complex in a laboratory includes co-precipitation, neutralization precipitation method, kneading, spray-drying technique, hot-melt extrusion, co-grinding/ milling, microwave technique and supercritical antisolvent technique [49].

1.2.7. Characterization of the Inclusion Complexes in Solid State

Characterization techniques implemented for the determination of the inclusion complex in this research is briefly discussed below:

1.2.7.1. Powder X-Ray Diffraction (p-XRD)

Powder X-Ray Diffraction (p-XRD) can be used to detect the inclusion complexation in the solid state. A comparison of the diffractogram of the guest compound, host molecule and complex is made. The difference in the patterns indicates the formation of complexes. Comparison is only valid if the host, as well as the guest molecule, is treated under the identical conditions. This is due to the fact that the process of preparation of the complex may change the crystallinity of the pure substances and this may cause differences in the diffraction patterns. The diffraction peaks are also used to determine

the chemical decomposition of the formed complex. The complexation changed the crystallinity of the drugs which alters the diffraction pattern [50, 51]. Formation of the crystalline complex in pXRD leads to sharpening of the existing peaks, appearance of new peaks or shifting of the certain peaks. On the other hand, formation of the amorphous complex leads to disappearance of a few peaks or peaks are less sharp than those of the pure molecule or physical mixture [52].

1.2.7.2. Infra-red (IR) Spectroscopy

Infra-Red (IR) spectroscopy can be used to identify the interaction between the cyclodextrin and the guest molecule in the solid state. The cyclodextrin band may change slightly upon the formation of the complex. If the fraction of the guest molecule is included, it can be easily masked by the band of spectrum of the cyclodextrin. IR is constricted to the drug molecule having some characteristic bands such as carbonyl or sulphonyl groups. IR spectral studies give information regarding the cleavage of hydrogen bonds due to the inclusion complexation, shifting the absorbance bands to a higher frequency [51, 53].

1.2.7.3. Thermo-analytical method: Differential Scanning Calorimetry (DSC)

Thermal analytical methods help in determining the thermal change before the degradation of cyclodextrin. The change as melting decomposition, oxidation, and polymeric transition of the guest molecules indicates the formation of the complexes

[51]. For analysis by these techniques, the guest molecule must have a characteristic melting or boiling temperature below about 300°C, the temperature at which cyclodextrin decomposes. The effect seen in the DSC thermograms may be appearing, disappearing and/or broadening of the peaks. The weak interaction between the drug and the excipients is represented by small shifts in the endothermic peak. The changes in heat flow with respect to temperature are recorded by DSC. The DSC is useful as a quantitative tool for characterization [50, 54].

1.2.7.4. Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) is used to study and compare the surface morphology or the microscopic aspects of pure cyclodextrin, pure drug and the formed inclusion complex. The difference in the morphology/ crystallization state of the CD complexes compared to pure CD represents the formation of the inclusion complex [51].

1.2.8. Characterization of the Inclusion Complexes in Liquid State

Nuclear Magnetic Resonance (NMR) Spectroscopy

Nuclear Magnetic Resonance (NMR) Spectroscopy research implements the use of 1D-^{1}HNMR (proton NMR) and 2D-NMR ROESY (Rotating frame Over-Hauser Effect Spectroscopy) as the ultimate characterization tools to elucidate the mode of complexation of the formed inclusion complexes. Along with information concerning the

formation of the inclusion complex, NMR also gives the information about the direction of the penetration of the guest molecule inside the cyclodextrin cavity. As the environment around the hydrogen atoms in the cavity changes resulting from complexation with the guest molecule, there is a change in chemical shifts in the peak of cyclodextrin. It is expected that there is a significant change in the chemical shift of the H-3 and H-5 atoms of the cyclodextrin directed towards the interior if the complex is formed. The H-1, H-2 and H-4 atoms which are located towards the exterior of the cavity only show small changes in chemical shifts [51, 55]. A chemical shift in peaks can be observed for both the cyclodextrin and guest molecule. Similarly ^{13}C-NMR, ^{15}F-NMR, ^{19}F-NMR, ^{31}P-NMR and 2-D-NMR like COSY, TOCSY, and ROESY can be used as methods for detection of complexes as well as elucidating the mode of complexation [56].

1.2.9. Advantages of Cyclodextrin Inclusion complexes

CD is mainly used as a complexing agent in order to increase the solubility, stability and bioavailability of drug molecules [57]. The complexation helps in improving the physicochemical properties of the existing drug molecules. Some of the major benefits associated with forming cyclodextrin inclusion complexes is outlined below:

1.2.9.1. Enhancement of Solubility

Inclusion complexes between drug molecule and cyclodextrins can increase the solubility of poorly water soluble drugs. The formed complex will have the hydrophobic functional group towards the interior of the cavity and hydrophilic functional groups towards the

exterior of the cavity which remains exposed to the environment. This makes the drug-cyclodextrin complex water soluble [31, 32, 58].

1.2.9.2. Enhancement of Bioavailability

Complexation reduces active recrystalization of drugs, which may help to increase their aqueous solubility [50]. The release of the formulation from the dissolved drug governs absorption of the orally administered drugs. When drug is complexed with cyclodextrin, enhancement of the solubility occurs, hence dissolution rate and absorption are enhanced as well. Cyclodextrins also increase the permeability of hydrophopic drugs by making drug available at the surface of the biological barrier such as skin, mucosa, etc. In the case of hydrophilic drugs, CD increases the drug permeability by direct action on mucosal membranes and enhances drug absorption and bioavailability [31, 59].

1.2.9.3. Improvement of stability

Cyclodextrin complexes help to improve chemical, physical and thermal stability of drugs [50]. When the drug molecule gets complexed inside the cyclodextrin cavity, it is difficult for the reactants to react with the guest molecule inside the cavity [27]. This prevents the drug molecule from degradation that occurs via oxidation, hydrolysis, chemical reaction, radiation or heat, photodecomposition, etc. This helps to improve the shelf life of the drug molecule. Cyclodextrin complexes insulate the drug molecule at the molecular level and thus insulates them against various degradation processes [58].

1.2.9.4. Reduction in drug irritation

Drugs, which are irritants to the mucus membrane of GIT and skin, are complexed with cyclodextrins to minimize the irritation [37, 38]. Complexation keeps the local irritancy of the drugs below the threshold level. As the complex gets dissociated and the free drug is released, absorption occurs simultaneously so the free drug concentration level always remains below the threshold that might be less irritating to the mucosa [59].

1.2.9.5. Taste masking of bitter API's by formulation of inclusion complex

Cyclodextrins are sweet, non-toxic cyclic oligosaccharides obtained from starch [60]. The complexation of the drug molecule with cyclodextrin helps to mask the bitter taste of the drug as well as any unpleasant odor [61, 62]. The functional groups that are responsible for causing the bitter taste are hidden inside the cavity and remain hidden from the sensory receptors of taste when complexed with cyclodextrin [55, 63]. Cyclodextrin is capable of masking the bitter taste by either decreasing its oral solubility upon ingestion or decreasing the amount of the drug particles exposed to taste buds [64]. The possible mechanism may be that the CD entraps the bad tasting molecule (inclusion complex formation), impeding its interaction with the taste buds, or the CD interacts with the gate-keeper proteins of the taste buds, paralysing them [60]. The resulting complexes have no or a little bit of taste and odor and are easily accepted by patients, especially pediatric populations.

Some of the bitter drugs that were taste masked by the inclusion complexation approach are shown in *Table 1.2*.

Table 1.2: Various complexing agents used for taste masking of the bitter drugs [61, 62]

Drug	Category	Dosage form	Complexing Agent Used
Zinc acetate dehydrate	Recover zinc deficiency		Anethol-β-cyclodextrin complex and saccharin
Carbapentane citrate	Local anaesthetic	Oral liquid	Cyclodextrins
Ibuprofen	NSAID	Solution	Hydroxypropyl β-cyclodextrin
Gymnema sylvestre	Anti-diabetic	Oral liquid	β-cyclodextrin,
Dioscin	CVS disorders		β-cyclodextrin,
Benexate HCl	Anti -Ulcer	Granules	β-cyclodextrin,
Metronidazole Benzoate	Anti-bacterial		γ -cyclodextrin
Hexitidine	Anti-bacterial		β-cyclodextrin
Zipeprol	Anti tussive		β-cyclodextrin
Guaiacol	Anti diarrhetic		β-cyclodextrin
Levosulpiride	Anti-psychotic		β-cyclodextrin

1.3. Cyclodextrin based oral drug delivery systems for pediatric patients

The oral route is the most popular and convenient route for designing drug delivery systems. Multifunctional characteristics of cyclodextrins (higher dissolution rate and taste masking ability when combined with oral drug delivery approach) enable the development of an effective formulation for pediatric patients [65-67].

1.3.1. Increase in Oral Bioavailability resulting from inclusion complex

Cyclodextrin, when complexed with a drug molecule, increases its solubility [68, 69]. Hence, there is an increase in oral bioavailability resulting from the cyclodextrin complexation. Previous reviews [70-73] suggest that bioavailabilty of the cyclodextrin

complexes and uncomplexed drug molecules were equivalent, however the absorption rate was much faster from the cyclodextrin based oral dosage forms.

1.3.2. Masking of bitter taste and reducing irritation of oral formulations

When the drug-cyclodextrin inclusion complex is formed, a smaller amount of free drug is available at the taste receptor sites. Most of the drug is enclosed inside the cavity which may help in masking the objectionable taste of the oral formulations [74]. CD complexation based oral formulations were also found to decrease the drug induced local irritation of GIT, thus modifying the time of drug release during GI transit [75].

1.3.3. Increase in mucosal drug permeability resulting from inclusion complex

Because of the formation of the inclusion complex, free drug that is available at the absorptive mucosal surface increases [76, 77]. Cyclodextrin complexation provides uniform absorption, enhancing mucosal drug permeability. This also enhances the drug activity upon oral administration. CD complexes enhance the dissolution rate of poorly water soluble drugs delivered via buccal or sublingual mucosa and have been used for oral formulations administered by the sublingual and buccal route [78-80].

1.3.4. Safety concerns for using cyclodextrins complexes in oral formulations

In regard to formulating drug-cyclodextrin inclusion complexes, relative safety and efficacy in terms of complexation, cost and acceptance needed to be considered. The reviews [38, 81] show that hydroxyl-propyl ß-cyclodextrins have a better safety profile as

compared to native ß-cyclodextrins and other parent cyclodexrtrins. Due to the lack of absorption of CDs through the GIT, all CDs are considered practically non-toxic for oral administration [82]. Also the safety profile depends on the drug dose used in drug-CD complexes. The Unites States Pharmacopoeia (USP) and National Formularies (NF) provide information concerning the optimum use of ß-CDs for forming drug complexes to be used in oral formulations. Modified CDs, such as HP ß-CD, can be used when their specific properties are required in formulations.

1.4. Alternative forms of Cyclodextrin based oral drug delivery systems for pediatric patients:

1.4.1. Limitation with conventional oral dosage forms for pediatrics

Swallowing of the solid oral dosage form is one of the greatest challenges in optimizing pediatric medications for oral drug delivery [83]. Pediatric patients may suffer ingestion problems as a result of underdeveloped muscular and nervous control [84]. Alteration of the oral tablets to develop a formulation suitable for children is always tried.

Crushing tablets, mixing them with food and/or water making the medication suitable for easy administration to children have been tried [85]. However, the problem with this approach is the slow rate or extent of drug absorption. Cutting the tablets into small pieces is another common practice in administering pediatric medicine. The problem with this practice is the considerable dose variation. It can induce toxicity in some cases with

the drugs having a very narrow therapeutic index [86]. A preparation of pharmaceutical oral suspensions is another approach to ease the administration of the medicine to the pediatric population. However, the primary concern with this is in regard to the stability of the formulations. Oral liquid formulations are more reliable as ready to use preparations for children but they are less stable than solid dosage forms and the bioequivalence with solid oral dosage forms is not assured [87].

These are the problems most commonly encountered during the administration of the medicine to children. This highlights the need for the development of new formulations that are easy to administer to the pediatric population and capable of maintaining the therapeutic plasma drug concentrations. An alternative oral drug delivery approach is the orally disintegrating tablet which is suited for children who are unable to swallow solid oral dosage forms such as tablets and capsules [88].

1.4.2. Orally Disintegrating Tablets

Orally Disintegrating Tablets (ODTs) are tablets designed to dissolve within one minute in the presence of saliva. The primary advantage is that no water is needed to swallow the medicine [89]. ODTs can be considered as a useful alternative for geriatric and pediatric populations who have difficulty swallowing tablets and capsules [90]. Orapred ODT® (ODT of prednisolone) has been effective in pediatric patients with asthma and allergic conditions [88]. The taste of the bitter drugs are usually difficult to mask in oral liquid products Delysm® (dextromethorphan hydrobromide oral suspension). The cyclodextrin-drug complex based ODT products minimizes the bitter taste of the formulation and

enhances the permeability for absorption through the oral mucosa and bioavailability. Zofran ODT ® (Ondansetron ODT) is used for the treatment of nausea and vomiting in pediatric population [88].

1.4.2.1. Advantages of ODTs

The major advantage for ODTs is that they provide the convenience of solid oral dosage forms (tablets and capsules), at the same time they also allow ease of swallowing as found with liquid oral formulations (suspensions and solutions) [91].

Other advantages include, water or other liquids are not required to swallow the medication, easily disintegrates into saliva within few seconds, pleasing taste, accuracy in dosing compared to liquid oral formulations and rapid onset of action because of increased rate of absorption and dissolution. [89, 92]

1.4.2.2. Formulations of ODTs

Selection of active pharmaceutical ingredients (API's) is one of the most important parameters to consider. The API should have low dose, small molecular weight and adequate solubility, non-ionized and should be absorbed via the oral mucosa. Excipient selection is important for the immediate disintegration of the tablets and masking the bitter taste of the API's. Disintegrants, binders, glidants, lubricants, sweeteners, flavors, taste making agents are some of the main excipients in the preparation of ODTs [93]. There are various technologies used for the formulation of ODTS that have been

patented. WOWTAB®, ORASOLV®, DURASOLV®, EFVDAS®, FLASHTAB® (main approach is conventional tablet processes with modifications), ZYDIS®, LYOC®, QUICKSOLV® (main approach is freeze drying method) and FLASHDOSE® (main approach is floss formation) are some of the patented technologies [92]. Some of the marketed ODTs and their manufacturing technologies, and major advantages are given below in *Table 1.3*.

Table 1.3: ODTs in market, name of patented ODTs technologies, their basis [89, 94, 95]

Active Ingredients	Local Brand Name	Category	Manufacturing Technology	Technological basis
Loratadine	Claritin	Antihistaminic	Zydis®	Lyophilization
Mirtazapine	Remeron	Antidepressant	Orasolv®	Compressed tablets
Olanzapine	Zyprexa	Antipsychotic	Zydis®	Lyophilization
Ondansetron	Zofran	Antiemetic	Zydis®	Lyophilization
Risperidone	Risperdal	Antipsychotic	Zydis®	Lyophilization
Zolmitriptan	Zomig	Antimigraine	DuraSolv®	Compressed tablets

Most of the above mentioned ODTs are for adolescents and adults. There are some ODTs that have been formulated for pediatric patients such as Children's Tylenol® Meltaways. These are grape punch or bubble gum flavored tablets, designed for children and may be chewed or allowed to melt in the mouth [88].

1.4.2.3. Direct Compression method for preparing ODTs:

This is the simplest and most cost effective tablet manufacturing technique. Direct compression is the most favored method for formulation of ODTs. The index of good compression for oro-dispersible tablets is determined by the factors such as dimensions, compressibility, powder flow ability, lubricity, etc [96]. There are many medications which have potential to benefit children but are not available since the formulation which is appropriate for them has not been developed. New alternatives or drug delivery technology that suits the pediatric population are continuously being researched. The ability to ease the administration of pediatric medications through ODTs can ease medication therapy for pediatric patients. Modified ODTs (drug-cyclodextrin inclusion complex based ODTs) are among one of the innovations in drug delivery technology that can have substantial impact on the pediatric medicine delivery technology.

1.4.2.4. Drug-cyclodextrin inclusion complexes based orally disintegrating tablets (Modified ODTs)

CD complexes to be used in oral formulation makes it suitable for children by masking the bitter and obnoxious taste [29]. Orally disintegrating tablets comprising of drug-CD complexes along with other excipients are ideally suited for pediatric patients who are unable to swallow tablets that are usually bitter in taste. The drug-cyclodextrin complex also enhances the permeability of the oral mucosa [84] which is the absorption site for the ODTs which rapidly increases the absorption rate and hence bioavailability.

This approach makes the formulations acceptable to children by

a. Eliminating the bitter taste of the medication through formation of the drug-CD inclusion complex.
b. Enabling the easy administration of the medicine without swallowing through development of orally disintegrating tablets.

1.5. Response Surface Methodology (RSM): A statistical tool for optimizing formulations

Response Surface Methodology (RSM) is a statistical method that allows us to investigate the interaction and relationships between the independent variables with one or more responses [97]. This provides the idea concerning the shape of the response surface we are investigating. This method is useful in finding the optimal process setting and helps to make the product or manufacturing process robust (insensitive to external influences) [98]. The main purpose for RSM is to find the optimum response. The other purpose for RSM is to evaluate how the response changes to a given desirable direction by adjusting the process or design variables.

The statistical method enables one to optimize the responses (dependent variables) which are influenced by various factors (independent variables). An experiment is conducted with a series of tests, called runs, where independent variables are changed in order to determine the reasons for change in the response or dependent variables. Utilizing this method, operation variables are evaluated that may or may not have significant effect in the response [99, 100].

In RSM, response can be represented graphically, either in a three-dimensional space or as counter plots which mainly helps in visualizing the shape of the response surface. Hence, the function of $f(x_1, x_2)$ is plotted vs. the levels of x_1 and x_2 as shown in *Figure 1.8a-b*. [101]

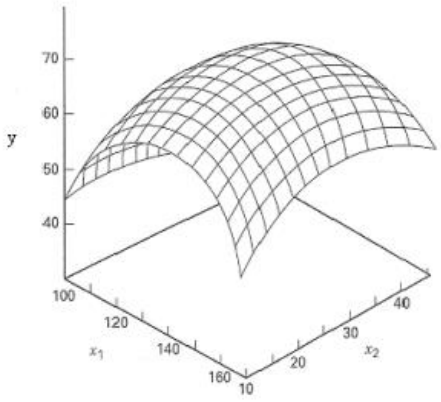


Figure 1.8a: Response Surface Plot

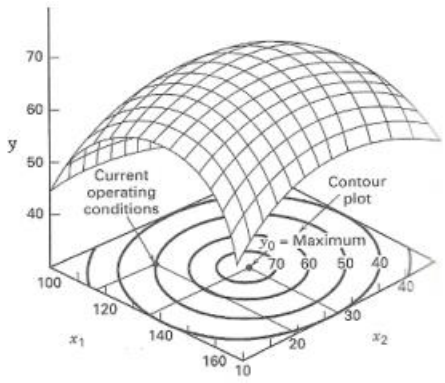


Figure 1.8b: Contour Plot

In *Figure 1.8a*, each value of x_1 and x_2 gives a y-value. This 3D-graph showing the response surface from the side is a response-surface plot. *Figure 1.8b* is the contour plots and it can show contour lines of x_1 and x_2 pairs that have the same response value *y*.

1.5.1. Central Composite Design (CCD) based RSM for optimization of process variables

In order to employ RSM in experimental optimization, it requires an experimental design to fit the mathematical function and to evaluate the quality of the fitted model and its accuracy. The central composite design is the symmetrical second order experimental design most utilized for the optimization of the manufacturing process and process

variables in pharmaceuticals [102]. The CCD consists of factorial points, central points and axial points. It develops through sequential experimentation. Basically, they are first order (2^k) designs augmented by additional center and axial points which helps in estimating the parameter of the second order model [103].

The design consists of three types of components: factorial design components, axial components and center point components. The factorial design component of CCD is 2^k factorial where k is the number of factors or independent variables. Each of the variable is taken at two levels representing high and low numeric value. The coding of the levels are done as -1 and +1 for low and high numeric values respectively. The geometrical representation of the factorial can be assumed as square in which each corner represents the interaction of the factors. Therefore, four interactions are to be evaluated when processing two variables to determine their significance in final response [104].

The axial components of CCD are the points that are equidistant from the center of the square formed for the factorial design. The radius α determines the geometry of the design region. If α is 1 it represents the square design geometry. As the value of α increase, the axial point extends beyond the faces of the square and the design region becomes more spherical. The α-value is calculated from the equation: $\alpha = (2^k)^{1/4}$, where k is the number of processing variables in the factorial design. Thus for 4 interactions or 2 variables/factors $\alpha = 1.41$ [104, 105]

The central point component is the average of the high and low value in the factorial design. The central point or the zero point is the region where the optimum conditions are met [104].

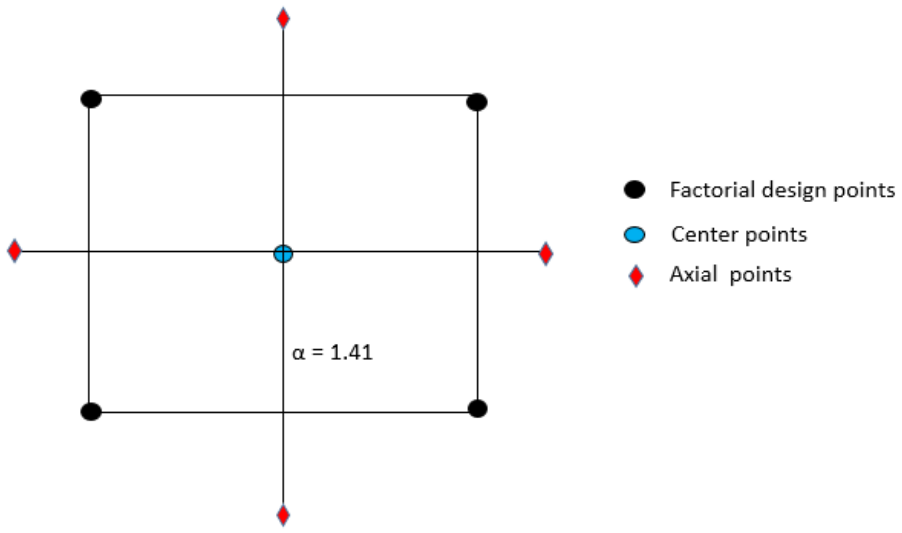


Figure 1.9: Layout of Central Composite Design (CCD) for 2 variables at 2 levels

The selection of process variables using CCD as Design of Experiments (DoEs) helps determine which factor may or may not have an important effect in the final response [99]. The use of CCD design has been limited to optimization for 2 variables because its efficiency is very limited for higher number of variables. However, box-behnken design offers more flexibility in terms of optimizing higher numbers of process variables and in designing the response surfaces. They are 3^k factorial designs accompanied by other block designs [106].

1.5.2. Application of CCD based RSM in ODTs formulation

RSM based optimized design helps in reducing the cost of the expensive manufacturing process and to solve process associated problems. RSM generates the large amount of information from the small number of experiments and also gives the wide range of

possibilities of in evaluating the interaction effect between the process variables in the response. [97]

The optimization of concentration of the superdisintegrants and diluents are the important steps in the development of the ODTs formulation [107]. This process variable using RSM implies the evaluation of hardness, disintegration time, dissolution profile, swelling index etc. in ODTs formulations [108, 109]. In this study hardness, disintegration time and dissolution of the tablet are evaluated as responses to determine the effect of various concentrations of superdisintegrants and diluents.

Chapter 2

Significance of thesis research

Dextromethorphan HBr (DXM HBr), an antitussive agent, is most commonly used as cough supressant among the pediatric population, alone or in combination with other expectorants, to produce the significant effect. It is a non-prescription cough medication available as conventional tablets, logenzes, liquid and syrups. However, its availability as an effective solid oral dosage form is limited, especially in children. The drug is extremely bitter in taste due to the presence of the amino moiety in its molecular structure and this leads to poor patient adherance in pediatrics. Most of the marketed formulations of DXM HBr for children are available as oral liquid formulations (syrups and suspensions). Stability, dosing problems and patient non-adherance are the key issues with oral liquid formulations for children.

A cyclodextrin based drug delivery system for DXM HBr utilizes cyclodextrin as the carrier material that has the ability to form water soluble drug-cyclodextrin inclusion complexes. This inclusion complex alters the physico-chemical characteristics of the drug molecule to overcome its undesirable properties. The 2-Hydroxy propyl ß-cyclodextrin (2- HPßCD) has multifunctional characteristics. These include masking the bitter taste of

the oral formulations, increasing oral bioavailability and increasing the mucosal drug permeability resulting from inclusion complex. The bitter taste of DXM HBr remains unmasked or difficult to mask in oral liquid preparations. Hence, 2- HPßCD - DXM HBr inclusion complex will minimize the bitter taste of DXM HBr in the formulation while enhancing the permeability for absorption through the oral mucosa.

Orally disintegrating tablets (ODTs) are designed to disintegrate within one minute in the presence of saliva. They will be best suited for pediatric populations who are unable to swallow conventional tablets of DXM HBr which is usually bitter in taste. These ODTs are rapidly absorbed through the oral mucosa because the tablets are comprised of DXM HBr -2- HPßCD inclusion complex that enhances the permeability of the oral mucosa.

This work is primarily directed towards formulating the orally disintegrating tablets of DXM HBr acceptable to childern by using a three-step approach:

i. Prepare a DXM HBr -2- HPßCD inclusion complex which minimizes/eliminates the bitter taste of DXM HBr preventing the interaction between drug and taste receptors while increasing the oral absorption by enhancing the permeability of the oral mucosa;

ii. Develop an orally disintegrating tablets comprised of DXM HBr -2- HPßCD inclusion complex that enables the easy administration this medication to pediatrics without swallowing;

iii. Use response surface methodology for the optimization of process variables to investigate the relationship between factors and response which explores the design space for formulating ODTs with desired response.

Chapter 3

Development and Optimization of Dextromethorphan HBr-2-Hydroxy Propyl ß-Cyclodextrin Inclusion Complex Based Orally Disintegrating Tablets Using Response Surface Methodology

Saugat Adhikari[1], Kenneth S. Alexander*[1]

[1]College of Pharmacy and Pharmaceutical Sciences, The University of Toledo Health Science Campus, Toledo, OH, 43614

KEYWORDS: Dextromethorphan HBr, 2-hydroxy propyl beta-cyclodextrin, inclusion complex, orally disintegrating tablets, response surface methodology, central composite design

Figures: 19

Tables: 13

***Corresponding Author**

Kenneth S. Alexander, Ph.D.
Professor
Department of Pharmacy Practice
The University of Toledo HSC, College of Pharmacy and Pharmaceutical Sciences
3000 Arlington Ave. (MS #1013)
Toledo, OH 43614
Phone: 419.383.1988
Fax: 419.383.1950
Email: kenneth.alexander@utoledo.edu

3.1. ABSTRACT

The focus of this present investigation was to access the utility of various characterization techniques in the evaluation of Dextromethorphan HBr (DXM HBr) inclusion complex with 2-Hydroxy propyl ß-cyclodextrin (2-HPßCD). This techniques confirms the formation of the inclusion complex and explores the mode of complexation between DXM HBr and 2-HPßCD. It also predicts the ability of 2-HPßCD to mask the bitter taste of DXM HBr and explain its taste masking mechanism. In aqueous solution, the inclusion complex was studied utilizing the phase solubility method. The solubility of DXM HBr increased as a function of 2-HPßCD concentration. The solubility profile was classified as A_L type: indicating the formation of a 1:1 stoichiometric inclusion complex. In solid state, the inclusion complex was prepared using lyophilization (freeze drying technique) and characterized by Differential Scanning Calorimetry (DSC), Fourier Transform Infrared (FT-IR), Scanning Electron Microscopy (SEM), powder X-ray Diffraction (pXRD), proton nuclear magnetic resonance (^{1}HNMR) spectroscopy and 2D-NMR rotating Over Hauser effect spectroscopy (ROESY). FT-IR showed no interaction between DXM HBr and 2-HPßCD and confirmed the formation of the complex. DSC and SEM studies further confirmed the inclusion complex formation. pXRD analysis indicated that the crystallinity of the inclusion complex reduced significantly. NMR spectroscopy elucidated the mode of complex formation. The subsequent incorporation of the inclusion complex into orally disintegrating tablets (ODTs) was done to develop the formulation. This results in patient adherence and convenience and enhances the dissolution rate by rapid absorption of drug through oral mucosa. Response surface

methodology with central composite design was employed in the optimization of the formulation factors, such as concentration of croscarmellose sodium (CCS) and microcrystalline cellulose (MCC), to obtain ODTs within the range of 3.5 to 5.5 kp hardness, 6.3 to 45 second disintegration time and 1.2 to 6.06 minutes mean dissolution time (MDT). The results indicated selected factors which have a strong influence on properties of the ODTs. The optimum concentration of CSS and MCC predicted by the model was 5.168 mg (2.5%) and 81.814 mg (40%), respectively for preparing a DXM HBr-2-HPßCD inclusion complex based ODT with a hardness of 4.5 kp, disintegration time of 10 seconds and MDT of 1.341 minutes. Thus, this approach exhibited the ability of masking the bitter taste of DXM HBr when complexed with 2-HPßCD, which resulted in ODTs formulations with improved patient adherence and acceptability.

3.2. INTRODUCTION

Although oral solid dosage forms as conventional tablets and capsules are most widely used because of dosing uniformity, ease of administration and high stability compared to liquid oral preparations. Many patients, particularly the pediatric population, suffer from ingestion problems and find the dosage form difficult to swallow [92, 110]. This can prolong the duration for treatment and cause patient non-adherance. Altering the conventional oral tablets to develop the formulation suitable for children has been tried. A phamaceutical oral suspension is another the another approach to ease the administration of the drug to pediatrics. However, stabilty of pharmaceutical suspensions have been a concern [111]. Orally disintegrating dosage forms that disperse or dissolve in

saliva within a minute and then swallowed without water can address the issues and is most suitable for children. ODTs have been investigated for their potential to increase the bioavailability by increasing absorption through oral mucosa and enhancing the dissolution rate [78, 79, 112].

ODTs are generally prepared by a direct compression method since it is the simplest and most cost effective method for production [113]. Selection of the excipients is one of the important parameters that governs the properties of ODTs. Disintegrants, binders, glidants, lubricants, sweetners, flavors, taste masking agents are excipients mainly used in its preparation. The solubility of diluents affects the dissolution and disintegration mechanism [114]. The concentration of the disintegrant and diluent is the key factor that affects the disintegration time and dissolution process. The optimization of the tablets disintegration time can be achieved by determining the optimal ratio and concentration of disintegrants and diluents. Below the critical concentration, disntegration time is inversely propotional to the disintegrant concentration and above it disintegration time remains approximately constant [115]. Hence, optimization of the ratio and concentrations of the diluents and disintegrats in an ODTs formulation will impact their attributes.

Natural cyclodextrins are cyclic oligosaccharides having six (α-cyclodextrin), seven (ß-cyclodextrin) or eight (γ-cyclodextrin) glucopyranose units linked by α- 1,4 glycosidic bonds into the macrocycle [27, 116]. It has a hydrophilic outer face and a hydrophopic cavity. Due to the presence of primary and secondary hydroxyl groups and a hydrophobic cavity, cyclodextrins are able to form inclusion complexes with various drug molecules. The physiochemical properties of the guest molecule or the included substance are altered

upon complexation and these inclusion complexes are used for improving stability, solubility, dissolution rate and biovailability [35, 117]. They can also minimize the bitter taste of extremely bitter drugs by enclosing the functional group responsible for the bitter taste inside the cavity. Inclusion complexation with bitter tasting drugs prevents the interaction between the bitter tasting functional group of the drug molecule with the taste receptors [118]. ß-cyclodextrin is widely used for complexation due to its price availabilty and cavity dimension. The ß-cyclodextrin cavity size is suitable for drugs with a molecular weight between 200 to 800 g/mol [119]. However, its low water solubility is a barrier to its wider utilization. Since natural cyclodextrins have relatively slow solubilty in water and organic solvents, derivatives are synthesized by chemical modification of the hydroxyl group as 2- Hydroxy propyl ß-cyclodextrin (2-HPßCD) which improves their solubilty, ability to dissolve hydrophobic compounds and is well tolerated with reduced toxicity [38, 120].

Dextromethorphan Hydrobromide (DXM HBr) is an antitussive agent, used as a cough supressant. It is over the counter (OTC) medication and available as syrups, lozenges, gels but its availability is limited in children as solid oral dosage forms [121]. This dosage form has poor compliance with pediatric patients because of its bitter taste due to the presence of amino moiety in its structure and is difficult for swallowing [122]. The dosage form faces challenges to overcome the bitter taste of the DXM HBr and the lack of its ability to ease the administration of the dosage form to the pediatric population. Drug-cyclodextrin inclusion complex based ODTs can ease medication therapy for pediatric patients and offer a solution to overcome the above mentioned challenges. In this regard, modifed ODTs (drug cyclodextrin inclusion complex based ODTs) are

among one of the newer drug delivery technology innovations that have had a substantial impact on medicine delivery technology for pediatrics.

Response surface methodology (RSM) is the widely used statistical aapproach that enables one to optimize the responses (dependent variables) which are influenced by various factors (independent variables). RSM is based on the priciple of design of experiments (DoEs) and encompasses techniques for designing experiments, building models, evaluating the effect of factors, generation of the polynomial equation, mapping of the response over the experimental domain to search for the optimum formulations [123, 124]. This statistical method predicts the desirable optimum response from minimum number of experiments and is far more effective than conventional methods of formulating dosage forms [123]. Experiments were performed using two-factor, three level face-centered central composite design. This design explores the quadratic response surfaces and allows the development of a polynomial model. Central composite design offers to estimate second-order and third-order effects, to identify the inter-relationships between factors and to locate the response optima [125].

In this present investigation, Dextromethorphan HBr – 2- Hydroxy Propyl ß-cyclodextrin inclusion complex based orally disintegrating tablets were proposed. The inclusion complex minimizes the bitter taste of DXM HBr in formulation while enhancing the permeabilty for absorption through the oral mucosa. Oral mucosa is the site of absorption for ODT fomulations. These inclusion complex based ODTs best suits the pediatric populations who face difficulty in swallowing bitter tasting conventional tablets of DXM HBr. In this study, central composite design based response surface methodology was employed to investigate the effects of two independent variables (factors) (i.e.

concentration of diluent microcrystalline cellulose and concentration of superdisintegrant croscarmellose sodium) on hardness, disintegration time and mean dissolution time of ODTs and to find the optimal value for attaining the desired response.

3.3. MATERIALS AND METHODS

3.3.1. Materials

Dextromethorphan Hydrobromide monohydrate was purchased from Spectrum Chemical Mfg. Corp. (New Brunswick, NJ, USA). The 2-HPßCD (Kleptose HPB-parental grade) and mannitol (PEARLITOL® 200 SD) were obtained from Roquette America Inc. (Keokuk, IA, USA). The following materials were obtained from JRS Pharma (Rosenberg, Germany) and used as received: croscarmellose sodium (VIVASOL®), silicified microcrystalline cellulose (PROSLOV SMCC®- 90M) and sodium steryl fumarate (PRUV®). Orange Flavor powder was supplied from Monsanto Flavor/Essence Inc. (Montvale, NJ, USA). All other reagents and solvents used were of analytical grade. Deionized water was used throughout the experiments.

3.3.2. Phase solubility study

The phase solubility study was carried out according to the method previously reported by Higuchi and Connors, 1965 [45]. The phase solubility diagram was obtained at 37ºC in water (pH 7). An excess amount of Dextromethorphan HBr (200 mg) was added to 10

ml of water in screw-cap glass vials containing increased amount of 2-HPßCD (ranging from 0 to 0.025M). The vials were protected from light, sonicated for 10 min, placed in a thermo-shaker (Thermo Fisher Scientific, USA) and shaken at 500 rpm continuously at 37 ± 0.5ºC. The suspensions were shaken for 48 hours after which equilibrium was reached. After equilibrium attainment, the sample solutions were filtered through a 0.20 µm membrane filter (EMD Millipore®, Fisher Scientific) and appropriately diluted. The solubilized DXM HBr in various concentrations of 2-HPßCD solution was determined by an HPLC method described below and studies were performed in triplicate. Previous determinations showed that 2-HPßCD did not interfere with HPLC determination at the employed concentration [126].

The stability constant (Ks) was calculated from the phase solubility diagram, with the assumption of 1:1 stoichiometry (as the slope is smaller than 1), according to the equation [45]:

$$Ks = \frac{slope}{S_0(1-slope)} \quad \text{.....Eqn. (3.1)}$$

where S_0 is the DXM HBr solubility at 37ºC in absence of 2-HPßCD.

3.3.3. Preparation of the inclusion complex in the solid state

Lyophilized inclusion complex was prepared by dissolving exact amounts (1:1 molar ratios) of DXM HBr and 2-HPßCD in deionized water at room temperature (25±1ºC). The resulting solution was frozen and freeze-dried over 48 hours (LABCONCO, Freeze Dry System Freezone 2.5® MO, USA) at -50ºC and about 0.03 mbar. The inclusion

complex was milled and sieved through 60 mesh sieve and stored in a desiccator protected from light until further analysis. Physical mixture was also prepared by careful mixing of the exact amounts (1:1 molar ratios) of DXM HBr and 2-HPßCD and homogeneous blending in a ceramic mortar for 10 minutes, powder of both components previously sieved.

3.3.4. HPLC Analysis

Dextromethorphan HBr was quantified from the lyophilized inclusion complex and ODTs by using the High Performance Liquid Chromatography (HPLC) method. A HPLC (Waters Alliance 2695 separation module, Milford, MA) equipped with a Zorbax C18 column (250 X 4.6 mm, 5µm packing) and photodiode array (Waters 2998) detector was used for analysis. The column temperature was maintained at 25˚C. The mobile phase consisted of phosphate buffer (20mM) pH 3.0 (adjusted using ortho-phosphoric acid): acetonitrile (25: 75) and was pumped isocratically at the flow rate of 0.5 ml/min. Injection volume was set to 20 µl. The retention time of DXM HBr (λ = 278 nm) was found to be 8.3 minutes. Different calibration standards of DXM HBr were prepared in methanol. For the calibration curve, each standard was analyzed in triplicate and the average peak area was plotted against concentration. The drug content was determined quantitatively by plotting a calibration curve. The assay method was found to be linear in the range of 0-100 µg/ml with a correlation coefficient of 0.9998.

3.3.5. Analysis of drug content in the inclusion complex

Physical mixture and lyophilized inclusion complex was evaluated for content uniformity of DXM HBr. Powder equivalent to 10 mg of DXM HBr was accurately weighed and transferred to a 10 ml volumetric flask to which 10 ml methanol was added. The suspension was sonicated in the sonicator bath for 10 min. After complete dissolution, the appropriate dilution was made and the samples were filtered through a 0.20 µm membrane filter (EMD Millipore®, Fisher Scientific) and analyzed for content of DXM HBr using HPLC method described above.

3.3.6. In-vitro release study from the inclusion complex

The *in-vitro* release from the inclusion complex and physical mixture was performed by a horizontal shaker method [127] using phosphate buffer pH 6.8 as the dissolution medium at 75 oscillations/min and 37ºC. The physical mixture and inclusion complex equivalent to 10 mg of DXM HBr each was transferred into a beaker containing 100 ml of phosphate buffer and a 2 ml aliquot was withdrawn at 2, 4, 6, 8, 10, 15, 20 and 30 min. The medium was replaced with 2 mL of fresh buffer. The samples were filtered and analyzed for the amount of DXM HBr released into the medium by the HPLC method described above. The dissolution profiles were evaluated by dissolution parameters i.e. drug dissolved at 4 minutes (DD_{4min}) and dissolution efficiency at 10 minutes (DE_{10min}) calculated from the area under the dissolution curve as reported by *P. Costa et. al* [128].

3.3.7. Characterization of the inclusion complex

3.3.7.1. Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) measurements for pure DXM HBr, pure 2-HPßCD, 1:1 physical mixture and 1:1 inclusion complex were carried out using a DSC 822^{e} Mettler Toledo instrument (*Mettler Toledo* GmbH, Schwerzenbach, CH) fitted with a TSO801RO sample robot and a TSO800GCI Gas control attached to a Nitrogen gas cylinder. The DSC analysis studied the change in the rate of heat absorbed by DXM HBr after complexation with 2-HPßCD. A Star e software® V8.10 was used to obtain the scans. The samples (3-5 mg) were placed and sealed in aluminum pans using the Mettler MT 5 microbalance. The thermal behavior was studied by heating the sample from 25-150ºC at the rate of 10ºC/min and under a nitrogen flow of 20 ml/min, using an empty pan sealed as reference.

3.3.7.2. Fourier Transform Infrared (FT-IR) spectroscopy

FT-IR spectra of pure DXM HBr, pure 2-HPßCD, 1:1 physical mixture and 1:1 inclusion complex were obtained using a FTS 4000 FTIR spectrometer (Varian Excalibur Series UMA 600 FTIR, Digilab, USA) equipped with germanium crystal. Spectra acquisitions were performed directly in powder samples with the application of 64 scans at a resolution of 2 cm^{-1} over the range of 4000 to 400 cm^{-1}.

3.3.7.3. Powder X-ray Diffraction (pXRD)

Powder X-ray diffraction (pXRD) patterns of DXM HBr, 2-HPßCD, 1:1 physical mixture and 1:1 inclusion complex were collected using X-ray diffractometer (PANanlytical's X'pert Pro®, Tokyo, Japan) equipped with X'Celerator high speed detector and CuKα source with a voltage of 45 kV, and a current of 40 mA. The samples were crushed, placed in an aluminum sample holder, and packed smoothly using a glass slide. The diffractograms were recorded in the 2θ angle range between 5° to 50° and the instrument was operated with continuous scanning speed of 4°/min. Crystallinity was determined by comparing some of the representative peak heights in the diffraction patterns of inclusion complex with a reference. The results were evaluated using the X-Pert Data collector version 2.1 software.

3.3.7.4. Scanning Electron Microscopy (SEM)

The surface morphology of pure DXM HBr, pure 2-HPßCD, 1:1 physical mixture and 1:1 inclusion complex were examined by using Hitachi S-4800 High Resolution Scanning Electron Microscope (Hitachi High-Technologies Corp., Tokyo, Japan). The samples were fixed on a brass stub using double-sided tape and then made electrically conductive by spray coating in a vacuum with a thin layer of gold at 0.6 kV for 10 seconds. The photographs were taken at an excitation voltage of 5 kV and magnification factors of 400.

3.3.7.5. Proton Nuclear Magnetic Resonance (^{1}HNMR) Studies and 2D-NMR ROESY

The ability of 2-HPßCD to form inclusion complex with drug molecules in order to increase solubility, bioavailability, dissolution rate and taste masking have been studied in large extent [26, 32, 54]. Most important information are obtained on physico-chemical properties of drug and solid phase structure of the inclusion complex via X-ray analysis, thermal analysis, Fourier transform infrared spectroscopy, surface morphological studies etc. and have been reported in numerous research work by various authors [129-131]. Although these characterization techniques provide vital information on complex formation and changes in physiochemical properties, the exact mechanism of complexation could not be deduced. The use of ^{1}HNMR has proven to hold promises for such purposes. In this study, the ^{1}HNMR technique has been used as an important tool for investigating the mode of complexation of the most favored complexes and obtaining a better knowledge on interaction between guest and cyclodextrin molecules.

Pure DXM HBr and pure 2-HPßCD solutions (10mM) were prepared in DMSO-d_6 in 5 mm NMR tubes. DXM HBr and 2-HPßCD solutions were mixed in a 1:1 molar ratio in the 5 mm NMR tubes. All the samples were subjected to NMR analysis. The ^{1}HNMR spectra of the pure components and their respective mixtures were obtained at 295 K on a Varian Unity Inova 600 MHz instrument with a Penta probe. Typical acquisition parameters consist of sweep width of 8000 Hz, acquisition time of 3 seconds, and number of transients of 16. A Rotational Overhauser Enhancement SpectroscopY (ROESY) experiment for the detection of intermolecular nuclear Overhauser effects (NOEs) between DXM HBr and 2-HPßCD was acquired for the 1:1 molar ratio at 295K using the same probe. The ROESY spectrum consisted of a 2048 (t_2, complex) by 750 (t_1, real)

matrix covering a 4500-Hz sweep width. Gaussian weighting functions were used in both dimensions to improve the signal to noise ratio and zero filling to 4096 × 4096 was applied before Fourier transformation. The ^{1}HNMR spectroscopy (one dimensional-1D) and 2D NMR Rotational Overhauser Enhancement SpectroscopY (ROESY) was used as ultimate characterization tools for the elucidation of the mode of complexation of DXM HBr and 2-HPßCD that predicted the taste masking mechanism of the formed inclusion complex.

3.3.8. Tablet Compression of Inclusion Complex

3.3.8.1. Design of Experiments (DOEs)

The central composite design consists of imbedded factorial or fractional factorial design with center points that are augmented with the group of axial points (star points) that allows the estimation of curvature [132, 133]. In face-centered central composite design, star points are at the center of factorial design points or each face of the factorial space [133]. Two factors, three levels face centered central composite design was used to optimize the factors i.e. diluent microcrystalline cellulose, MCC (X_1) and superdisntegrant croscarmellose sodium, CCS (X_2) concentrations. The factors were evaluated at three levels high, medium and low (+1, 0, and -1) as shown in *Table 3.1*. Tablet hardness (Y_1), disintegration time (Y_2) and mean dissolution time-MDT (Y_3) formed the responses. These are the critical quality attributes that affect the performance of ODTs. A total of 13 experimental trials were designed by the software with five center points, four axial and four factorial design points shown in *Table 3.2*.

Table 3.1: Variables in Central Composite Design (CCD)

Independent variables (Factors)		**Design Level**		**Coded Level**
Croscarmellose Sodium – CCS, X_1 (mg)	A	0	low	-1
		6	medium	0
		12	high	+1
Microcrystalline Cellulose –MCC, X_2 (mg)	B	0	low	-1
		60	medium	0
		120	high	+1
Dependent Variables (response)				
Tablet hardness (Y_1) - kp				
Disintegration Time (Y_2) - sec				
Mean Dissolution Time-MDT (Y_3) - min				

Table 3.2: Design matrix of face centered CCD for ODT formulations

Formulation No.	Coded levels of Independent Variables	
	Factor X_1: CSS	Factor X2: MCC
F1	-1	+1
F2	-1	0
F3	0	+1
F4	0	0
F5	0	0
F6	0	-1
F7	+1	-1
F8	+1	+1
F9	0	0
F10	+1	0
F11	0	0
F12	-1	-1
F13	0	0

3.3.8.2. Tablet Manufacturing

All tablet formulations were manufactured by direct compression. The composition of the tablet formulations is displayed in *Table 3.3*. DXM HBr-2-HPßCD inclusion complex

was sieved through #40 mesh sieve before mixing. All other excipients were accurately weighed and mixed altogether with DXM HBr-2-HPßCD inclusion complex in a Turbulant® mixer (Chemi Pharm, 260 West Broadway, NY, USA) for 10 minutes. Sodium stearyl fumarate was added to the blend and lubricated for 5 more minutes in the mixer. The tablets were compressed by a single station tablet press machine (Emil Korsh Maschinen Fabrik, Berlin, Germany) using 0.375 inch round and flat punch and die set. The compression force was kept constant throughout the study. The targeted tablet weight (die volume) was kept constant around 200 mg. Formulations were prepared according to the matrix of the face-centered CCD varying the level of factors i.e. concentration of CCS (0, 6. 12 mg) and concentration of MCC (0, 60, 120 mg) as shown in *Table 3.3.*

Table 3.3: DXM HBr ODT formulations

Ingredients	**Weight[a] (mg)**
DXM HBr-2-HPßCD inclusion complex (Lyophilized powder)	10*
Croscarmellose Sodium (CCS)	0 / 6 / 12
Microcrystalline Cellulose (MCC)	0 / 60 / 120
Sodium stearyl fumarate	2
Orange flavor	2
Mannitol	q.s. 200

[a]Total tablet weight = 200 mg

* Inclusion complex powder equivalent to 10 mg of DXM HBr

3.3.9. Evaluation of prepared ODTs

3.3.9.1. Weight Variation

Weight variation for each batch of ODTs was assessed. Twenty tablets from each batch were individually weighed and average weight and standard deviation were reported.

3.3.9.2. Thickness

Preweighed 10 tablets from each batch were tested. A micrometer (Mitutoyo, Plymouth, MI, USA) was used to measure the average thickness and standard deviation was reported.

3.3.9.3. Friability

Twenty tablets of the formulation were weighed and measured in a roche type friabilator (Erweka, Germany). Rotation speed was set to 25 rpm for 4 minutes, and the tablets were re-weighed. The percentage friability was calculated using the equation:

$$\%\, friability = \frac{W_I - W_F}{W_I} \times 100\% \quad \text{.....Eqn. (3.2)}$$

where W_I and W_F are initial and final tablet weights, respectively.

3.3.9.4. Drug Content

Three tablets were weighed individually for each batch and crushed in a ceramic mortar. An accurately weighed quantity of powdered tablets (600 mg) was extracted with pH 6.8 phosphate buffer and the sample solutions were filtered through a 0.20 µm membrane filter (EMD Millipore®, Fisher Scientific) and estimated by HPLC method described previously. The studies were done in triplicate and average values and standard deviations were reported.

3.3.9.5. Hardness

Ten tablets of each batch with known weight and thickness were examined for tablet hardness using a hardness tester (type H1 T, Sotax, MA, USA). The average hardness and standard deviation for each batch were reported.

3.3.9.6. Wetting time and water absorption ratio

This was carried out using the method reported by *Bi et. al* [134]. A tissue paper of size 15 × 15 cm was folded twice and was placed in the petri dish (9 cm diameter) containing 6 ml of water. Tablet was placed on top of the tissue paper and time required for the water to reach the upper surface of the tablet was noted as wetting time. Experiment was performed in triplicate. The tablets were weighed before and after wetting. Water absorption ratio (R) was determined using the following equation:

$$R = \frac{W_a - W_b}{W_b} \times 100\% \quad \text{.....Eqn. (3.3)}$$

where W_b and W_a are the weights before and after water absorption respectively.

3.3.9.7. In-vitro Disintegration Test

In-vitro disintegration test was performed as per USP requirements for immediate release tablets. Six tablets were put in each tube disintegration apparatus (Erweka, Germany) and

the tablets were immersed in distilled water maintained at 37±1ºC. Times for complete disintegration of each of the tablets were recorded. The studies were done in triplicate and the average value and standard deviation were reported.

3.3.9.8. In-vitro Dissolution Test

ODTs were evaluated for dissolution behavior. Dissolution tests were performed as per USP requirements for immediate release dosage forms using USP II apparatus paddle method (AT 7, Sotax, MA, USA). Dissolution was carried out in 500 ml of phosphate buffer pH 6.8 at 75 rpm and 37±0.5ºC. The dissolution in pH 6.8 was chosen to simulate the pH conditions of saliva fluid. A 2 ml sample was withdrawn at 2, 4, 6, 8, 10, 15, 20, 30 minutes. It was then passed through a 0.20 µm Millipore filter and injected in the HPLC for estimation of DXM HBr into the medium. The experiment was performed in triplicate.

3.3.10. Statistical Analysis and Optimization

Statistical models with interaction terms were derived to evaluate the effect of the two factors (X_1 and X_2) on response variables: tablet hardness (Y_1), disintegration time (Y_2) and mean dissolution time-MDT (Y_3). Polynomial models were generated for all the response variables. In this study a linear model was used to determine the relationship between factors and response variables (Y_1) and quadratic models for determining the relationship between factors and response variables (Y_2) / (Y_3). Each experimental

response (Y) in the CCD model can be represented by an equation of the response surface represented by;

$$\mathbf{Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2}$$ (Linear Model)…… (Eqn. 3.4)

$$\mathbf{Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \beta_4 X_1^2 + \beta_5 X_2^2 + \beta_6 X_1^2 X_2 + \beta_7 X_1 X_2^2}$$ (Quadratic Model)

… (Eqn. 3.5)

Where, Y is the response variable, β_0 is an intercept, and β_1 to β_7 are regression coefficients, X_1 and X_2 are the factors studied. The polynomial equations from this optimization technique were used to predict the tablet hardness (Y_1), disintegration time (Y_2) and mean dissolution time-MDT (Y_3) values for ODTs. Comparison of predicted values for Y1, Y2 and Y3 with actual experimental values was used to test the validity of the response surface models.

3.4. RESULTS AND DISCUSSION

3.4.1 Phase Solubility Study

The phase solubility diagram was obtained at 37ºC by plotting the apparent solubility of DXM HBr against increasing concentration of 2-HPßCD as reported in *Figure 3.1*. It was observed that the solubility of DXM HBr from the complex increased linearly as a function of 2-HPßCD concentration, over the entire concentration range studied. The phase solubility profile (linear plot) was classified as A_L type. These A_L type curves indicate the formation of water soluble complexes between the substrate (DXM HBr) and the ligand (2-HPßCD) and a first order dependency of interactions on the 2-HPßCD

concentration [45]. This linear substrate-ligand correlation with a slope value less than 1 suggested the formation of first order soluble complexes, i.e. formation of 1:1 stoichiometric inclusion complex.

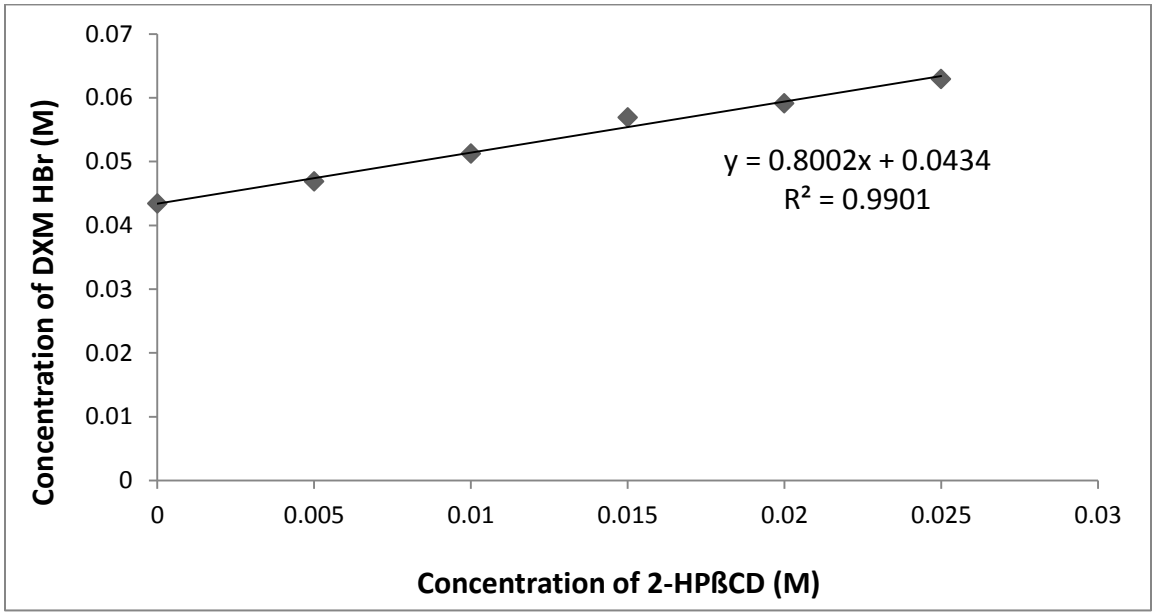


Figure 3.1: Phase solubility diagram of DXM HBr-2-HPßCD inclusion complex (n=3)

Note: Error bars are too small for visualization

The apparent stability constant (K_s), DXM HBr solubility (S_0), slope and correlation coefficient (r^2) of phase solubility diagram are given in *Table 3.4*. The calculated K_s value was dependent on the initial solubility of the drug (S_0). The K_s value of 1:1 complex was calculated according to the equation given by Higuchi and Connors, 1965 [45]. The K_s value reflects the favorable positioning of DXM HBr inside the cyclodextrin cavity demonstrated that hydrophobicity of the guest molecule and steric factors between guest and host molecules were responsible for these interactions [126, 135]. We found K_s value to be 92.3 M^{-1} and this indicates that the DXM HBr interacts strongly with 2-

HPßCD in water. Similar results were obtained by Marques et al. (1990) [126] and F. Veiga et al. (1996) [117]. In phase solubility studies for ß-cyclodextrin and methyl-beta cyclodextrin performed by Marques et al. the solubility curves shows almost similar patterns and K_s value of 68.9 M^{-1} and 82.7 M^{-1}, respectively, indicating the strong interaction between guest and host molecule [126]. F. Veiga et al. (1996) calculated K_s of HPßCD in water to be 144.8 M^{-1} [117]. G. Zingone reported increased K_s value for warfarin at pH 7.4 followed by the decreased K_s value at lower pH 1.2 [131]. They explained that the strength of complexation between drug and cyclodextrin depends on pH and is greater at pH 7.4 than the acidic pH of 1.2. We obtained a similar Ks value of 92.3 M^{-1} in water (pH 7) and results are almost coincident. This suggested that 2-HPßCD formed the stable inclusion complex in water (pH 7) with DXM HBr.

Table 3.4: DXM HBr solubility (S_0), slope, stability constant (K_s) and correlation coefficient (r^2) from phase solubility diagram

Medium	Water
S_0±SD	0.0434
Slope	0.8002
K_s (M^{-1})	92.30
r^2	0.9901

3.4.2. Drug Content in the inclusion complex

The actual drug content in the physical mixture and lyophilized complex was determined. The results are reported in *Table 3.5*. As seen in the table, both physical mixture and lyophilized complex showed a good agreement between theoretical and actual drug content.

Table 3.5: Drug content in the inclusion complex (% ± SD)

	Theoretical %	**% ± SD**
Physical Mixture	100	95.83±0.76
Lyophilized Complex	100	96.5±1.08

3.4.3. Characterization of inclusion complex

3.4.3.1. Differential Scanning Calorimetry (DSC)

The DSC curves for DXM HBr, 2-HPßCD, 1:1 physical mixture (C) and 1:1 lyophilized (D) DXM HBr-2-HPßCD inclusion complex studied are reported in *Figure 3.2*. DXM HBr showed the typical behavior of an anhydrous crystalline drug, exhibiting the sharp endothermic peak at 120.3ºC, corresponding to the melting point of the drug. The DSC curve of 2- HPßCD showed a very broad endothermal phenomenon between 55ºC to 110ºC, due to the release of water molecules [131]. The physical mixture of DXM HBr with 2- HPßCD showed the endothermic peak at 120ºC but with decreased enthalpy of reaction. The cyclodextrin dehydration peak was also present in the DSC curve for the physical mixture since the thermogram was the combination of the components analyzed separately. Thus, weak interaction between drug and 2- HPßCD can be postulated in such a system. The complete disappearance of the DXM HBr endothermic peak was observed for lyophilized DXM HBr-2-HPßCD inclusion complex indicating the encapsulation of drug molecule inside the cavity or formation of the amorphous complex or both [129]. The results obtained were in good agreement with those previously reported [136, 137]. Some evidence of inclusion complexation was obtained from thermal analysis.

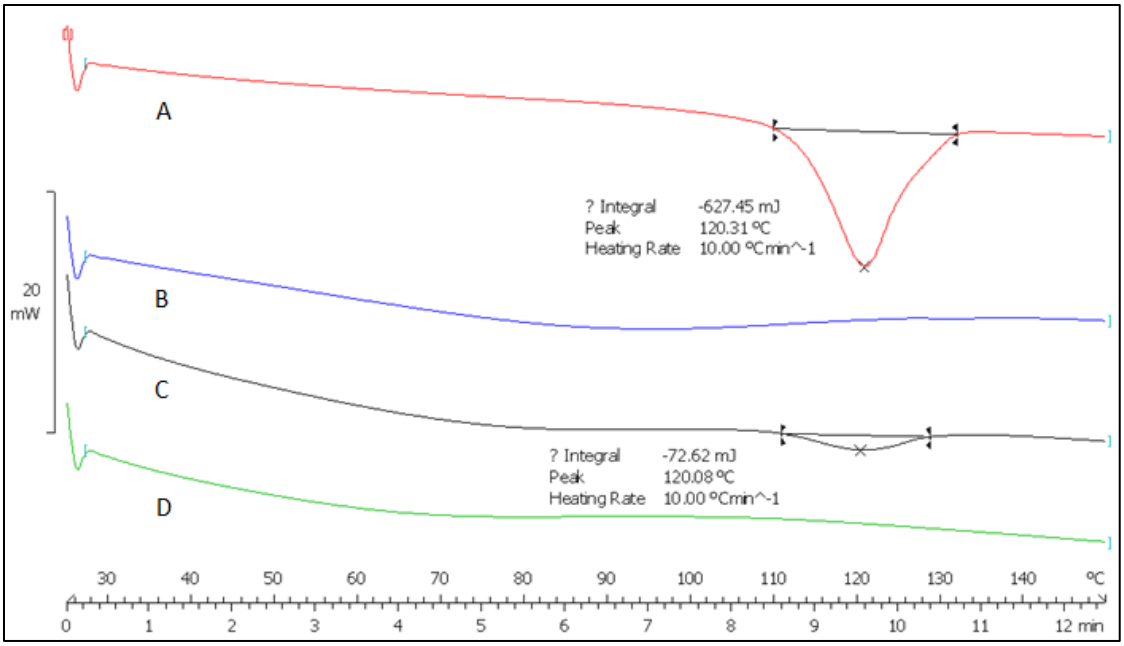


Figure 3.2: DSC thermograms of pure DXM HBr (A), pure 2-HPßCD (B), 1:1 physical mixture (C) and 1:1 lyophilized (D) DXM HBr-2-HPßCD inclusion complex

3.4.3.2. Fourier Transform Infrared (FT-IR) spectroscopy

FTIR spectroscopy investigated the functional groups of DXM HBr involved in the complexation providing supportive evidence of complex formation. The FTIR spectra of all the samples are presented in *Figure 3.3*. In DXM HBr spectra characteristic band of aromatic C-H stretch at 3277 cm^{-1}, C=C stretching at 1608 cm^{-1}, C-N stretching at 1445 cm^{-1} and methoxy (CH_3-O-) stretch at 2921 cm^{-1} was observed [138] and used to determine the interaction between 2-HPßCD and DXM HBr in solid state. FTIR spectra for 2-HPßCD showed O-H stretching at 3290 cm^{-1} [138]. Spectra for both the physical mixture and lyophilized complex did not show new peaks indicating that no chemical bonds were created in the formed complex however, shifting of the characteristic peak at 1445 cm^{-1} towards a lower wave number (1358 cm^{-1}) in lyophilized the inclusion

complex was observed. This suggests the formation of hydrogen bonds between the amino group of DXM HBr and the hydroxyl groups of the cyclodextrin cavity [129]. The amino group of DXM HBr is responsible for the bitter taste of the drug [24]. From these findings, it can be postulated that complexation of the bitter tasting amino functional group inside the cavity drastically reduces the bitterness of DXM HBr. In the physical mixture, characteristic peaks of DXM HBr were still detected but with low intensity which indicates that a weak interaction occurs between drug and cyclodextrin for forming the inclusion complex. The disappearance of the DXM HBr characteristic peak at 3277 cm^{-1}, 1608 cm^{-1} and 1445 cm^{-1} in the lyophilized complex can be attributed to inclusion of these functional groups inside the 2-HPßCD cavity. These findings are in full agreement with other authors [130, 139].

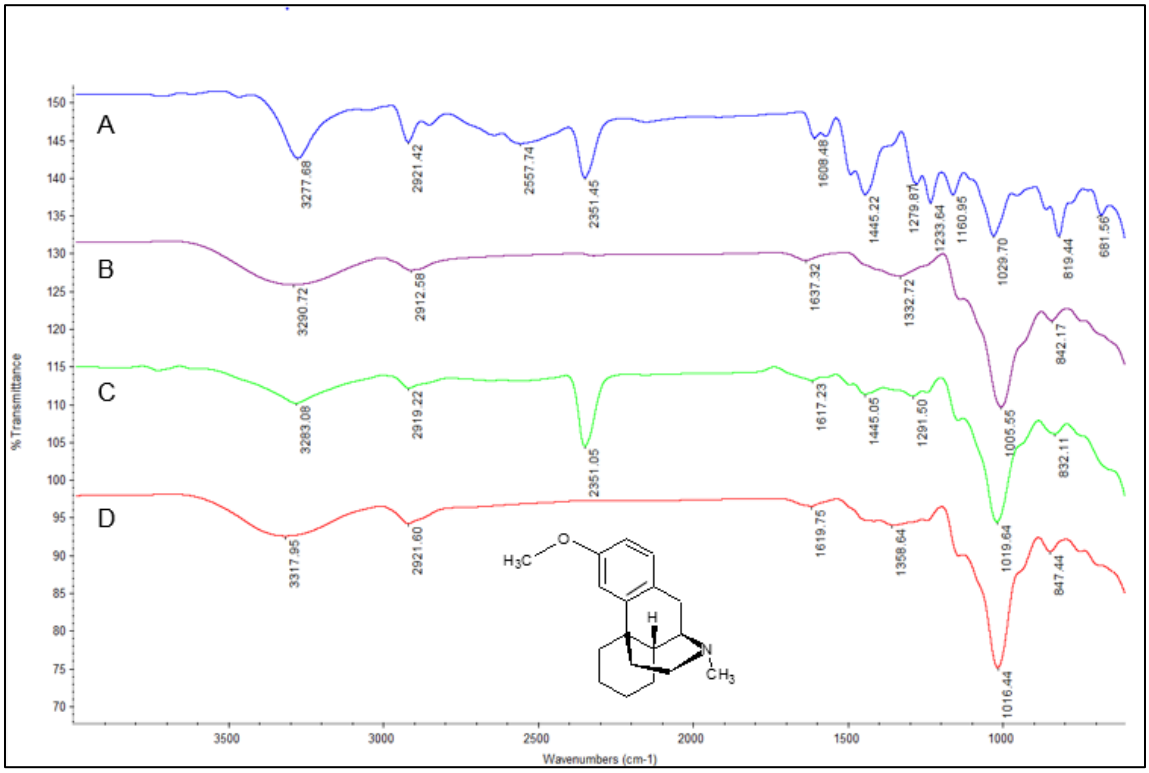


Figure 3.3: FTIR spectra of pure DXM HBr (A), pure 2-HPßCD (B), 1:1 physical mixture (C) and 1:1 lyophilized (D) DXM HBr-2-HPßCD inclusion complex

3.4.3.3. Powder X-ray Diffraction (pXRD)

The XRD pattern for the pure DXM HBr presented several diffraction peaks indicating the crystalline nature of the drug as shown in *Figure 3.4*. In contrast, 2-HPßCD was present in an amorphous form. The 1:1 physical mixture also exhibited a typical crystalline diffraction pattern but of less intensity compared to the diffraction pattern for the pure drug. This confirmed the presence of DXM HBr in its crystalline form in the 1:1 physical mixture and no inclusion complex was formed. It also showed a weak interaction between drug and cyclodextrin in the physical mixture confirming the DSC results. DXM HBr-2-HPßCD inclusion complex displayed diffuse diffraction patterns (identical to that of 2-HPßCD without drug peaks), suggesting the entirely amorphous nature of DXM HBr in 1:1 lyophilized complex. These results are attributed to the interaction between DXM HBr and 2-HPßCD indicating the possibility of complexation of DXM HBr inside the cyclodextrin cavity and the formed inclusion complex is amorphous in nature [129].

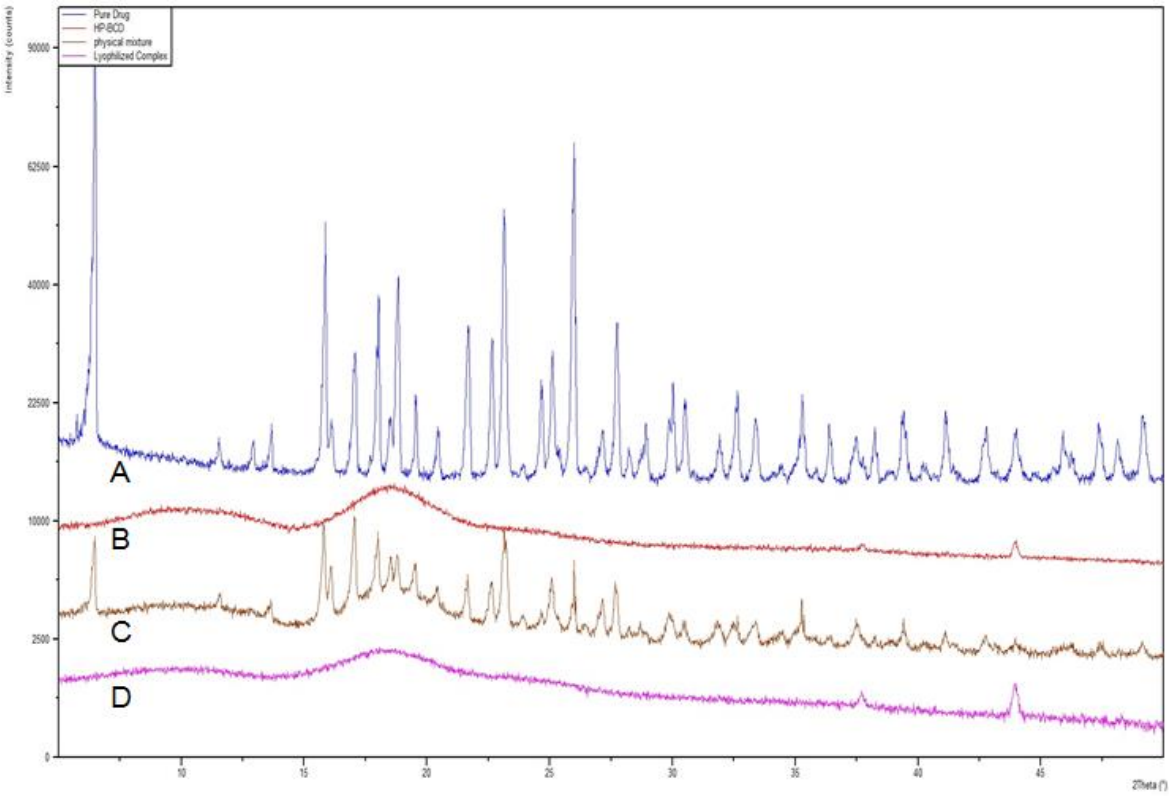


Figure 3.4: X-ray diffraction patterns of DXM HBr (A), 2-HPßCD (B), 1:1 physical mixture (C) and 1:1 lyophilized (D) DXM HBr-2-HPßCD inclusion complex

3.4.3.4. Scanning Electron Microscopy (SEM)

SEM microphotographs of the DXM HBr, 2-HPßCD, 1:1 physical mixture and 1:1 lyophilized DXM HBr-2-HPßCD inclusion complex are shown in *Figure 3.5*. DXM HBr appeared as irregular shaped crystalline particles while 2-HPßCD was a spherical particle with amorphous character. The 1:1 physical mixture consisted of a bulky particle (2-HPßCD) with characteristic DXM HBr crystals adhered on its surface. The surface morphology was evident of the presence of unmodified particles of 2-HPßCD covered by drug crystals and was clearly detectable in the microphotograph of the 1:1 physical mixture. Thus, no interaction took place in the physical mixture in the solid state.

However, a drastic change in the structural morphology of the lyophilized complex was seen and is indicative of the presence of the new solid phase confirming the formation of the inclusion complex. This also suggests that the new solid phase is the product of the homogenous distribution of the two components which could be responsible for the increased dissolution rate of the drug [127].

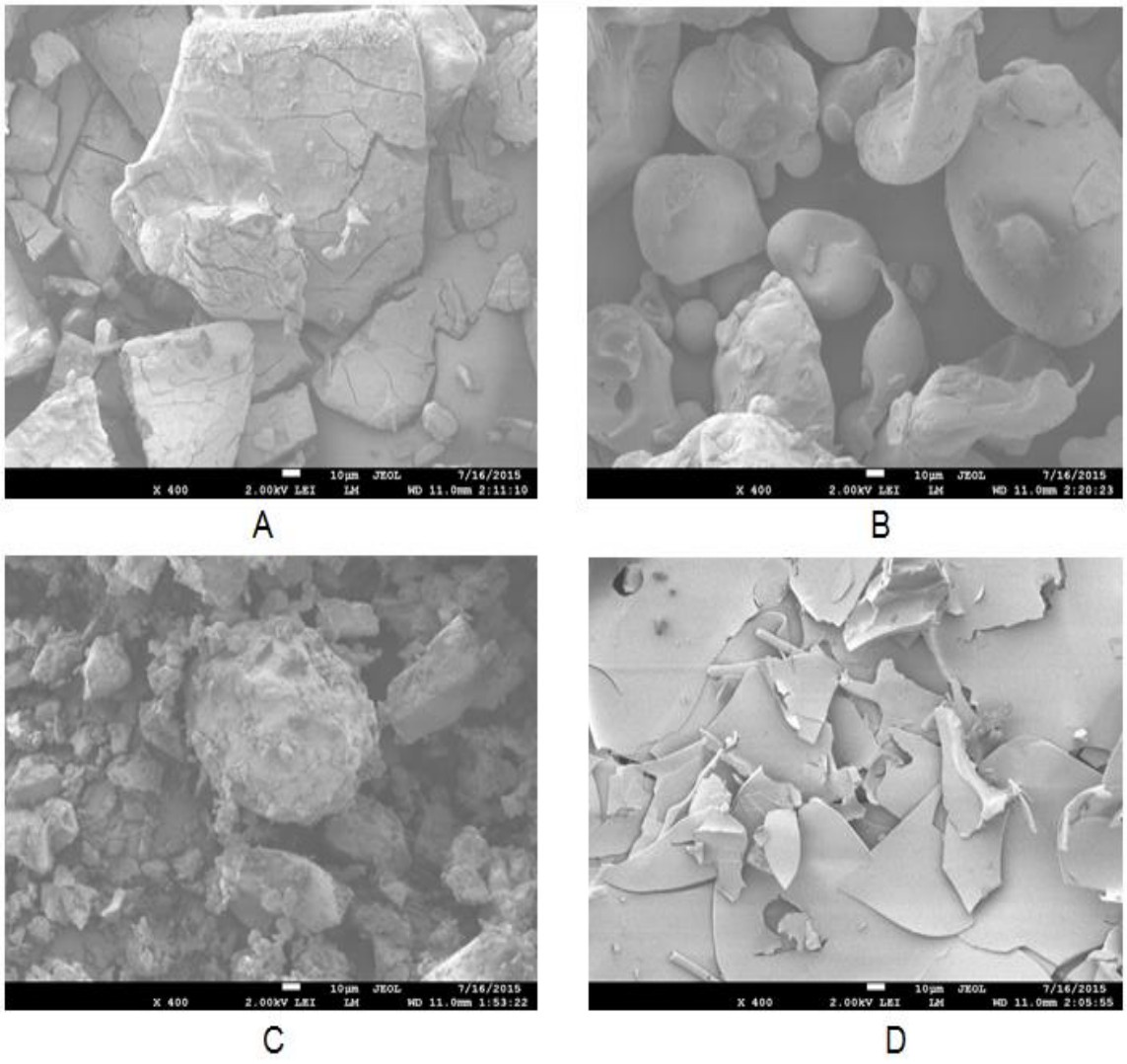
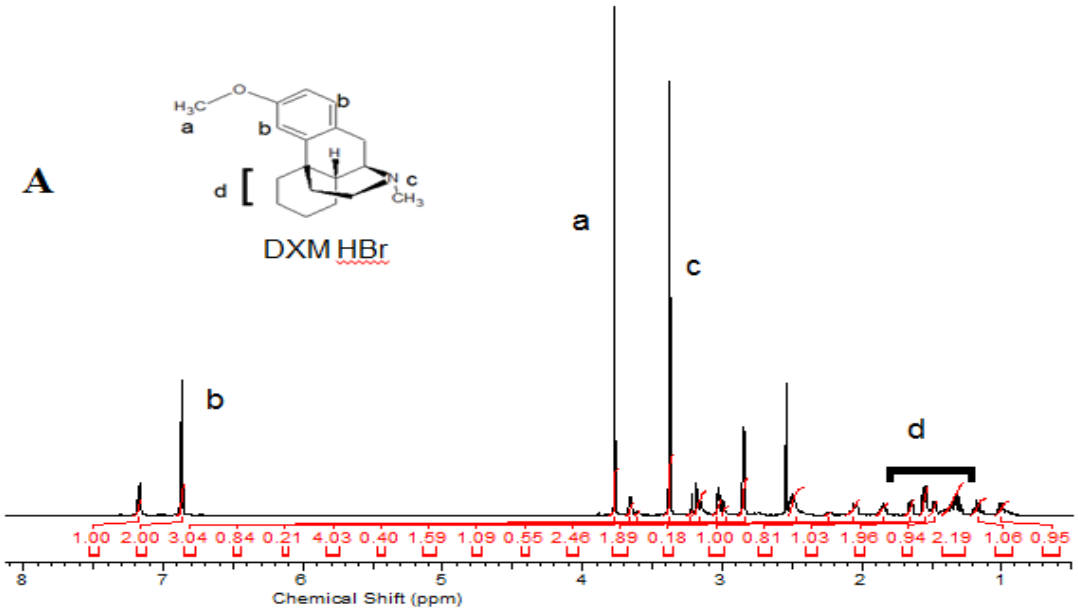


Figure 3.5: Scanning electron microphotographs of DXM HBr (A), 2-HPßCD (B), 1:1 physical mixture (C) and 1:1 lyophilized (D) DXM HBr-2-HPßCD inclusion complex

3.4.3.5. Proton Nuclear Magnetic Resonance (^{1}HNMR) Studies and 2D-NMR ROESY

Physico-chemical characterization studies such as *FT-IR*, thermal analysis, X-ray analysis and surface morphology studies indicate only nominal information concerning the formation of the complex. However, ^{1}HNMR studies relate to the functional group involved in the complexation and the chemical shift values in ^{1}HNMR depict the mechanism of complexation [56, 140]. The proton chemical shift between pure DXM HBr and its inclusion complex with 2-HPßCD was observed and compared to determine the interaction between the drug and 2-HPßCD. An amino-functional group is responsible for the bitter taste of DXM HBr [122]. The structural elucidation using ^{1}HNMR showed inclusion of the amino functional group inside the 2-HPßCD cavity which predicted the taste masking mechanism of DXM HBr when complexed with 2-HPßCD. Pure DXM HBr, pure 2-HPßCD and 1:1 DXM HBr-2-HPßCD lyophilized inclusion complex were analyzed for ^{1}HNMR studies (*Figure 3.6*).



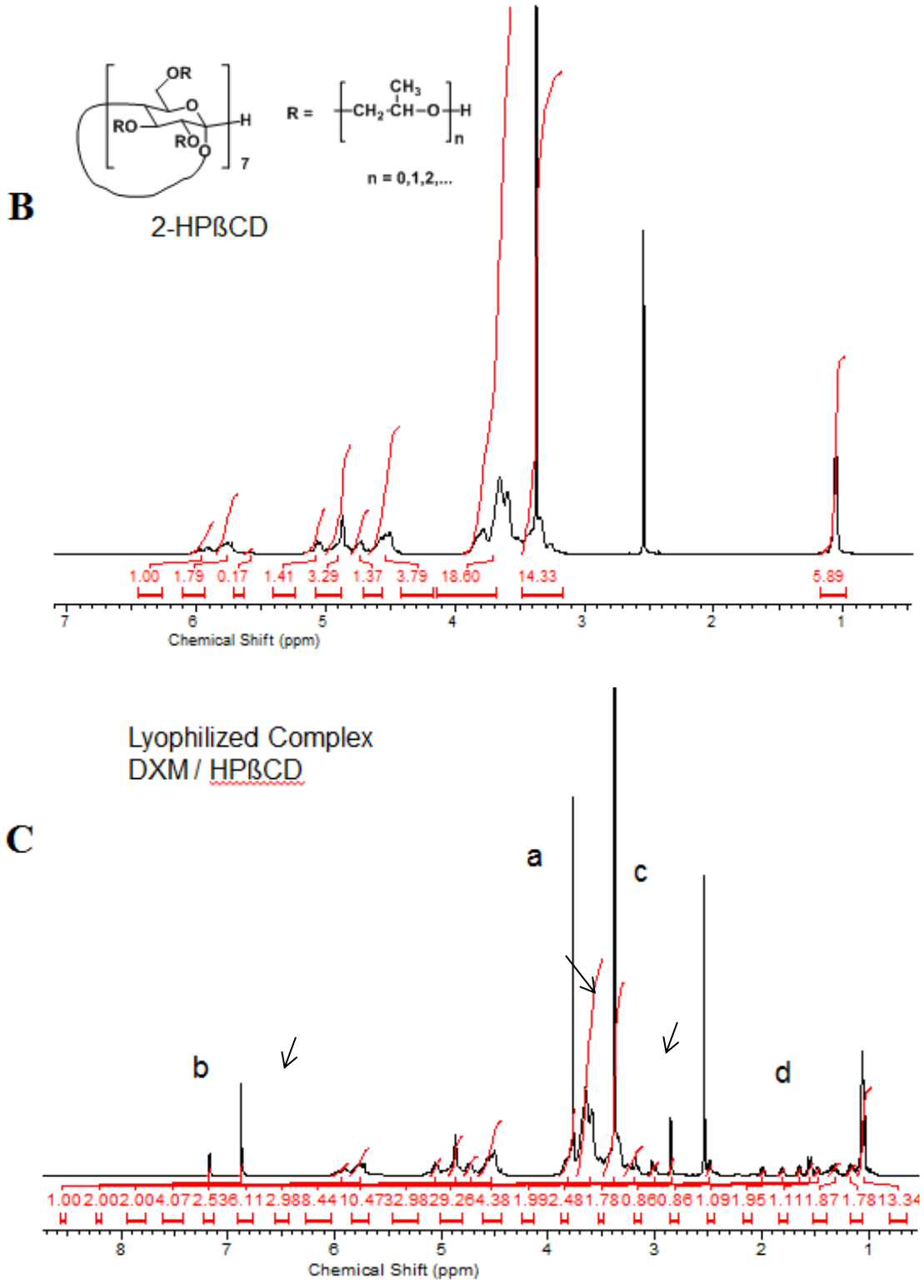


Figure 3.6: NMR spectra of DXM HBr (A), 2-HPßCD (B) and 1:1 lyophilized DXM HBr-2-HPßCD inclusion complex (C)

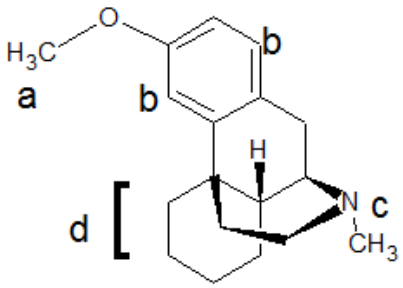


Figure 3.7: Structure of Dextromethorphan HBr with labelling of proton (denoted as a-d)

In ^{1}HNMR studies of the interaction between DXM HBr and 2-HPßCD, ^{1}H-chemical shift changes of DXM HBr protons resonance were analyzed (shown by arrow in *Figure 3.6c*). The chemical structure of DXM HBr with labelling of proton (denoted as a-d) is shown in *Figure 3.7* and corresponding chemical shift values of the protons are listed in *Table 3.6.*

Table 3.6: ^{1}H-chemical shift corresponding to DXM HBr in presence and absence of 2-HPßCD

DXM HBr proton	Chemical Shift δ (ppm)		Change in δ (ppm)
	$\delta_{(free)}$	$\delta_{(complex)}$	Δδ
a	3.77	3.77	0
b	6.90	6.89	-0.02
c	3.38	3.31	-0.07

The insertion of the DXM HBr molecule into the HP-ßCD cavity was clearly demonstrated by changes in ^{1}HNMR proton chemical shift values. There was no change in the chemical shift value of methoxy proton (proton a) while there was a significant change in the value of the tertiary amine (proton c) of DXM HBr (*Table 3.6*). These

results indicated that the tertiary amine group of DXM HBr becomes enclosed inside the HPßCD cavity. This can be well correlated with the FT-IR findings that postulated the complexation of the amino functional group inside the cavity.

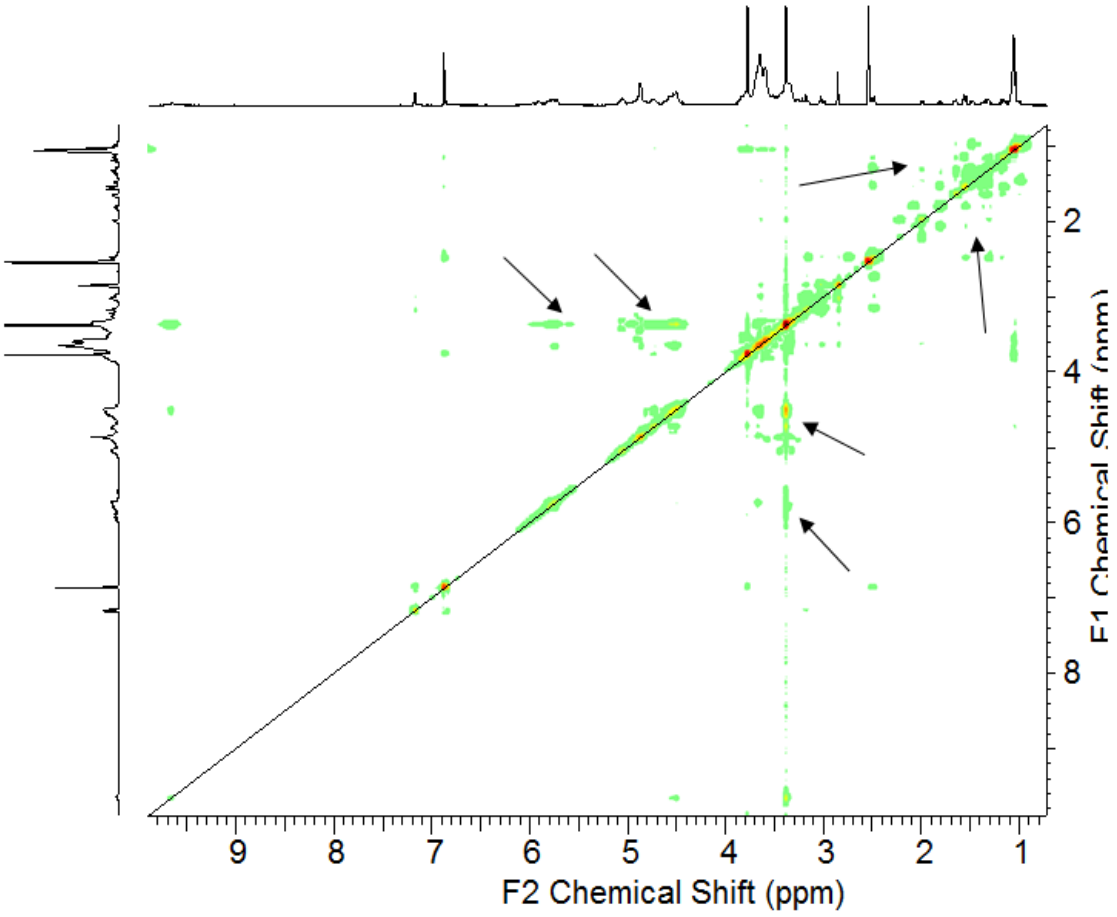


Figure 3.8: 2D-NMR ROESY plot of DXM HBr-2-HPßCD inclusion complex showing the intermolecular NOEs between protons of DXM HBR and H- protons of 2-HPßCD

In this study, the approach for preparing the drug cyclodextrin inclusion complex was to reduce or mask the bitter taste of DXM HBr and this complex will be used in the preparation of pediatric ODT fomulations. The ^{1}HNMR spectra results indicated the possible taste masking mechanism may be that the amino functional group of DXM HBr

gets enclosed inside the cavity and reduces/masks the bitterness. The postulated taste masking mechanism needed to be confirmed. The 2D-NMR ROESY (two dimensional) was carried out and serves the purpose to evaluate and confirm the mode of complexation and spatial arrangement between the host and guest atoms obtained from ^{1}HNMR studies. Cross-peaks of DXM HBr-2-HPßCD inclusion complex in 2D-NMR ROESY is shown in *Figure 3.8*. These cross peaks in two dimensional plot displayed the intermolecular Nuclear Over-hauser Effects (NOEs) between the amino protons (proton c) and H atoms of 2-HPßCD. Thus, 2D-NMR confirmed the result that was obtained with ^{1}HNMR which suggested the interaction between the amino group of DXM HBr with the hydrogen atom of 2HPßCD. Thus, we propose that the taste masking mechanism (shown in *Figure 3.9*), which predicts the complexation of the amino functional group, becomes enclosed inside the 2-HPßCD cavity and the bitter taste of DXM HBr is truly being masked.

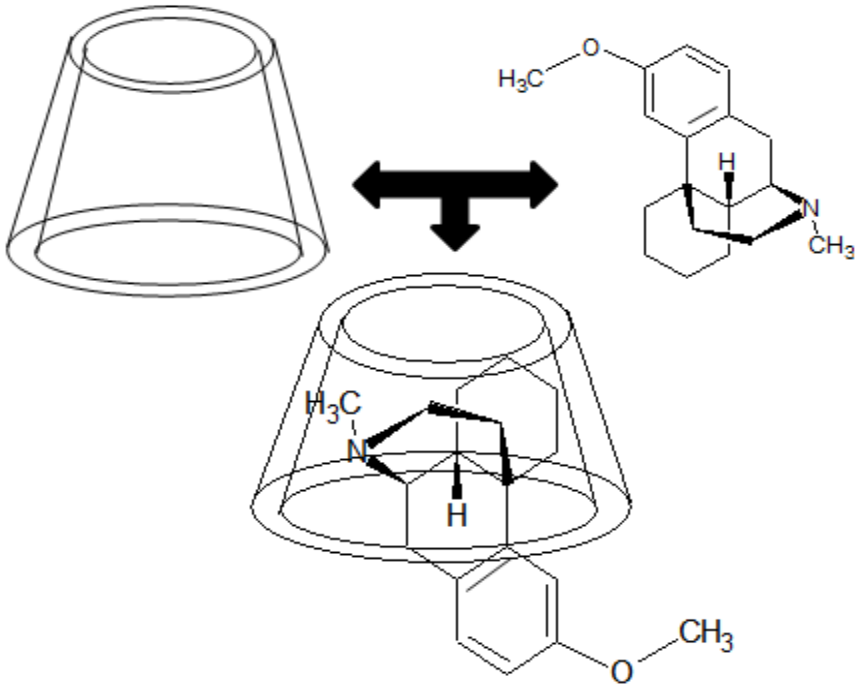


Figure 3.9: Proposed mechanism of complexation/ mechanism for taste masking of DXM HBr-2-HPßCD complex

3.4.4. Dissolution studies from inclusion complex

Dissolution profiles of pure DXM HBr, 1:1 physical mixture and 1:1 lyophilized complex are shown in *Figure 3.10*. All the formulations dissolved approximately 90% of the DXM HBr in 10 minutes. Dissolution parameters, dissolution efficiency after 10 minutes (DE_{10min}) and percentage of drug dissolved after 4 minutes (DD_{4min}) were measured for all formulations as illustrated in *Table 3.7*.

Table 3.7: Dissolution parameters for DXM HBr in pure, physically mixed and lyophilized form

	DXM HBr	DXM HBr:2- HPßCD inclusion complex	
		1:1 physical mixture ± SD	1:1 lyophilized complex ± SD
DD_{4min} (%)	43.67 ± 3.51	71.11 ± 8.00	91.58 ± 5.93
DE_{10min} (%)	56.08	67.27	83.502

SD- Standard Deviation

There were significant differences in the dissolution profiles for pure drug, physical mixture and lyophilized complex. Considering the DE_{10min} values, the dissolution rate of DXM HBr was increased in the order: pure drug < 1:1 physical mixture < 1:1 lyophilized complex suggesting that the dissolution rate was influenced by the lyophilization method used to prepare the inclusion complex. After 4 minutes, the percentage of pure drug dissolved was 43.67% however, the percentage drug dissolved from the physical mixture and lyophilized complex were 71.11% and 91.5% respectively, after complexation with 2-HPßCD. The increase in the dissolution of DXM HBr in the physical mixture and lyophilized complex is due to the high solubility of 2-HPßCD in water. This can be explained as the result of better wettability of the drug particles and solubilizing effect of

cyclodextrin [131]. Subsequently, the interaction between the DXM HBr and hydrophobic cavity of 2-HPßCD resulted in the formation of the readily soluble complexes [141]. A very high increase in the drug dissolution rate observed in lyophilized complex as compared to physical mixture is attributed to amorphization of DXM HBr in lyophilized complex and the amorphous form of the substance has a higher dissolution rate than its crystalline form [142].

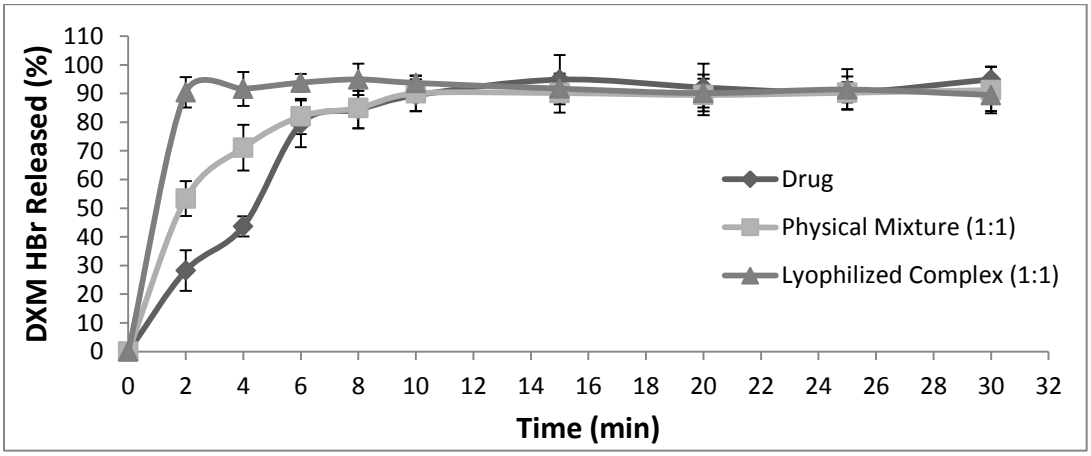


Figure 3.10: Dissolution profiles at pH 6.8 of pure DXM HBr (◊), 1:1 physical mixture (□) and 1:1 lyophilized complex (Δ)

3.4.5. Orally disintegrating tablets characterization and evaluation

Tablet formulations were prepared according to the design matrix shown in *Table 3.2* based on the formulae mentioned in *Table 3.3.* DXM HBr ODT's properties such as weight, thickness, friability, wetting time and water absorption ratio and drug content uniformity are shown in *Table 3.8*. All the tablet formulations met the requirements in terms of weight variation, thickness, friability, wetting time and water absorption ratio. Drug content uniformity ranged from 97.3% to 103.7% and was within the acceptable limits.

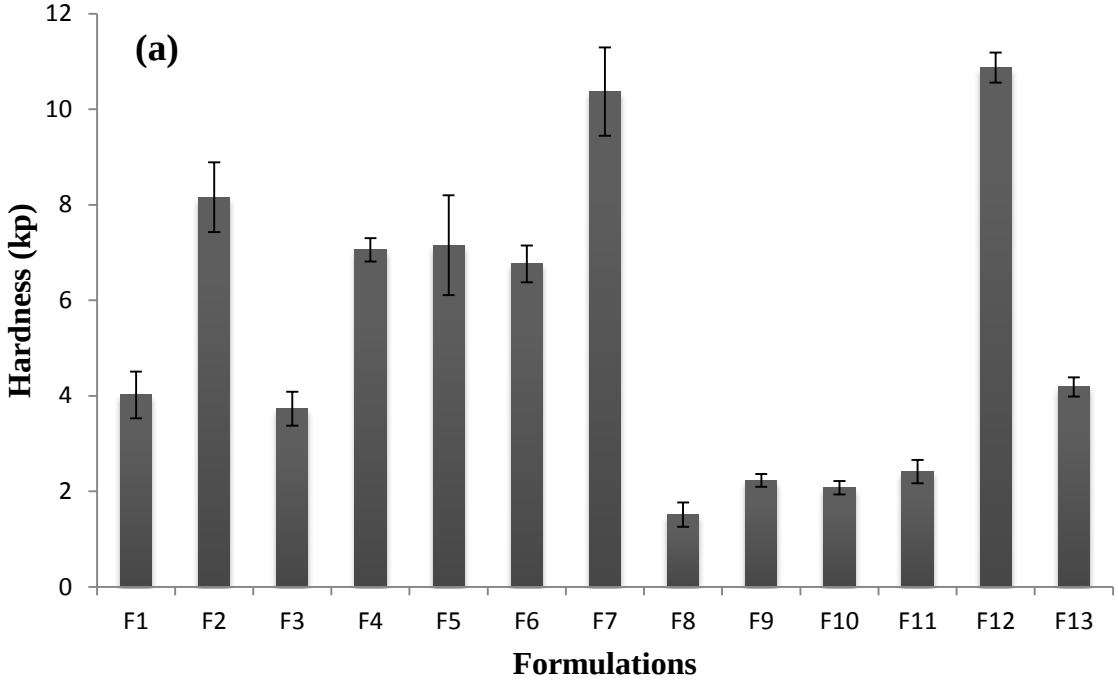
Table 3.8: Results of DXM HBr ODTs properties for each experimental run

Formulation No.	Weight (mg ± SD)	Thickness (mm ± SD)	Wetting time (sec ± SD)	Water absorption ratio (%± SD)	Friability (%)	Drug Content Uniformity	
						%	RSD
F1	200.85 ± 4.43	3.29 ± 0.014	4.41 ± 0.51	105.80 ± 5.63	0.929	102.11	2.61
F2	201.90 ± 3.40	2.50 ± 0.012	133.18 ± 7.44	58.25 ± 8.9	0.105	100.68	2.62
F3	200.55 ± 2.30	3.32 ± 0.018	4.20 ± 0.27	131.48 ± 2.97	0.965	100.91	1.16
F4	199.20 ± 1.67	2.56 ± 0.008	47.87 ± 5.49	56.38 ± 14.09	0.651	98.96	2.52
F5	200.45 ± 3.30	2.57 ± 0.013	51.51 ± 2.81	42.62 ± 3.34	0.571	102.93	1.92
F6	200.40 ± 4.00	2.44 ± 0.020	104.48 ± 4.89	17.13 ± 1.98	0.877	103.71	1.32
F7	201.10 ± 4.72	2.26 ± 0.017	131.28 ± 8.71	19.94 ± 8.09	0.601	98.89	1.21
F8	195.95 ± 1.57	3.94 ± 0.017	2.41 ± 0.41	156.47 ± 9.33	1.062	97.33	0.96
F9	201.10 ± 3.24	3.20 ± 0.012	5.42 ± 1.16	80.15 ± 3.95	0.542	99.41	2.30
F10	197.40 ± 2.39	3.24 ± 0.008	7.00 ± 0.51	96.98 ± 3.05	0.962	98.77	2.92
F11	201.90 ± 3.72	3.22 ± 0.082	11.67 ± 3.95	84.55 ± 3.43	0.525	100.74	2.89
F12	201.85 ± 2.30	2.28 ± 0.020	214.29 ± 7.46	18.41 ± 6.01	0.354	100.95	2.75
F13	201.45 ± 3.34	2.93 ± 0.013	18.14 ± 3.19	75.26 ± 7.57	0.661	102.47	1.08

SD - Standard Deviation

The DOE was adopted to optimize the critical formulation factors based on their effect on characteristic responses that affect the performance of orally disintegrating tablets. The characteristic responses include tablet hardness, disintegration time and mean dissolution time (MDT).

From this study, tablet hardness varied from 1.5 to 10.8 kp, disintegration time ranged from 6.3 to 230 seconds and mean dissolution time (MDT) from 1.20 to 6.06 min (*Table 3.9 and Figure 3.11a-c*).



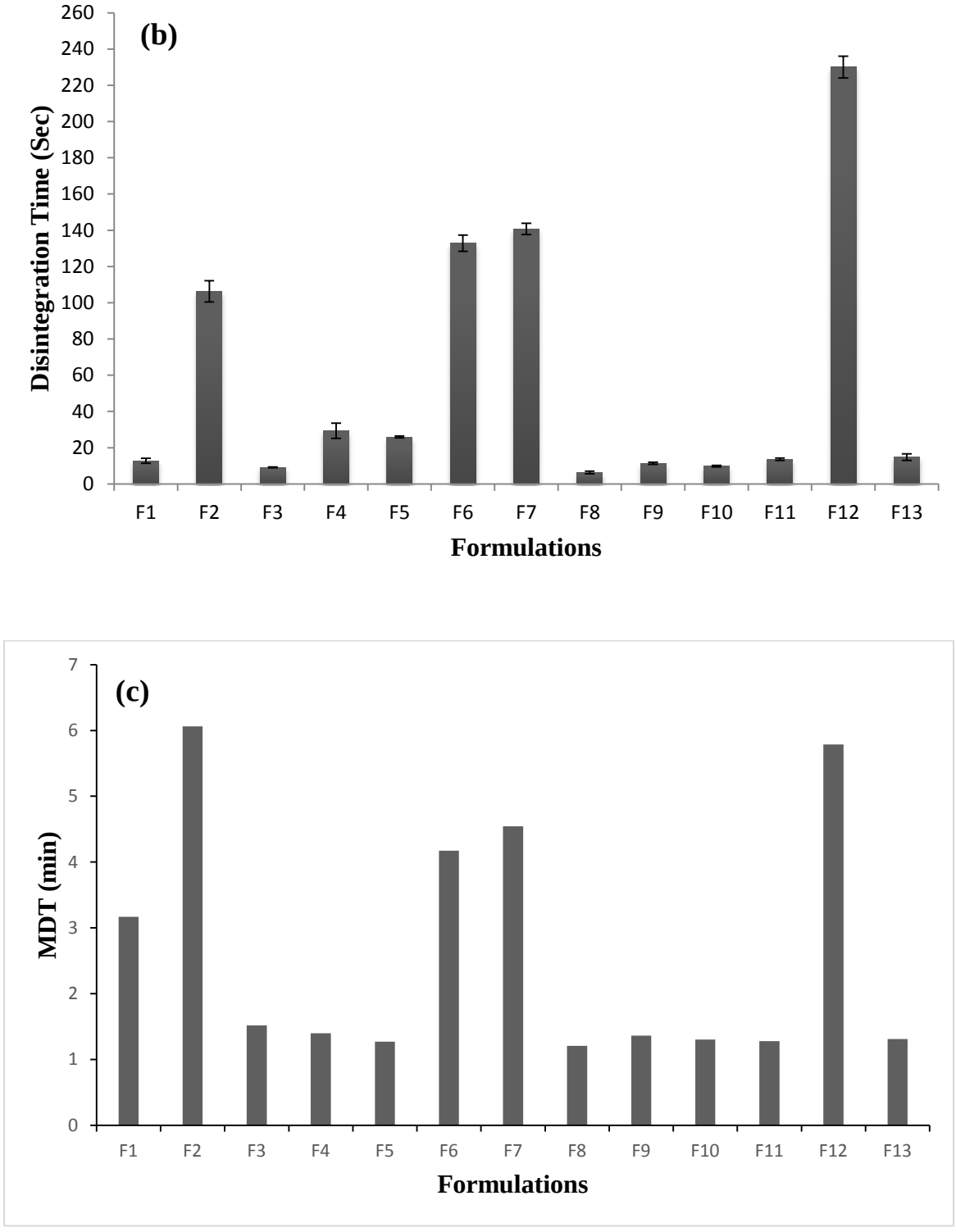


Figure 3.11: Effect of formulation factors (CSS and MCC) on characteristic response variables of DXM HBr ODTs

In *Figure 3.11a,* F7 (formulation with low CCS concentration and without MCC) and F12 (formulation without CCS and MCC) were found to have maximum hardness when compressed into tablets. Mannitol was used as filler and these formulations had high concentrations of mannitol due to the absence of CCS and MCC. These results are in agreement with *Sherif Badawy et. al* who reported an increase in tablet hardness and compactability with an increase in the concentration of mannitol [143].

In *Figure 3.11b,* Formulations F2, in the absence of CCS showed an increase in disintegration time. Formulation F6 and F7, in the absence of MCC, showed similar effects. In addition, the formulation F12 (in absence of both MCC and CSS) also increased the disintegration time. This shows that the concentration of CCS and MCC combined or alone has a strong influence on the disintegration time. Formulation F1 with a high concentration of MCC and an absence of CCS, showed a disintegration time within one minute. This can be attributed to the disintegrant properties of MCC at higher concentrations [144]. All other formulations in the presence of CCS and MCC showed disintegration of tablets within one minute. The disintegration mechanism of CCS and MCC can be explained by their ability to increase the porosity of tablets, pulling water into the pores reducing the physical bonding between the particles (wicking) and cause the particle to swell and break up from within (swelling) [134]. It concludes from the above findings that tablet disintegration is affected by swelling ability, hydrophilicity, porosity and inter-particle force.

Table 3.8 shows the time required for complete wetting of all formulations. F2 (formulation having medium concentration of MCC) and F6, F7, F12 (formulations without MCC) showed an increase in wetting time as compared to the other formulations. These findings can be attributed to the properties of MCC to decrease the wetting time by means of wicking and swelling of the tablet matrix by widening the tablet pores [134, 144].

The relationship between the wetting time and disintegration time is shown in *Figure 3.12*. A linear correlation (R^2 = 0.9515) was seen suggesting the strong relationship between them and that wetting is the important step for the disintegration process.

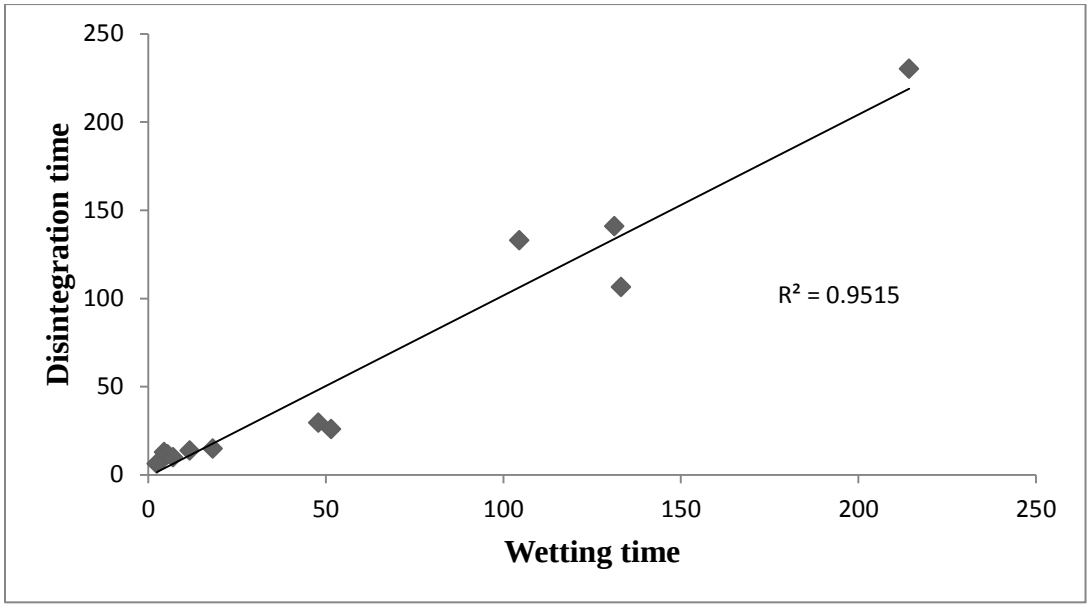


Figure 3.12: Relationship between wetting time and disintegration time

Table 3.8 illustrates the water absorption ratios for all formulations. F1, F3 and F8 have high MCC concentrations; similarly F6, F7 and F12 are without MCC in the formulations. All other

formulations have intermediate concentrations of MCC. The water absorption ratio increased as a function of increasing MCC concentration. This is shown in *Figure 3.13*, the higher absorption of water decreases the disintegration time (compare *Figure 3.13* with *Figure 3.11b*) as a result of a strong linear relationship (R^2 = 0.9515) between wetting time and disintegration time (*Figure 3.12*).

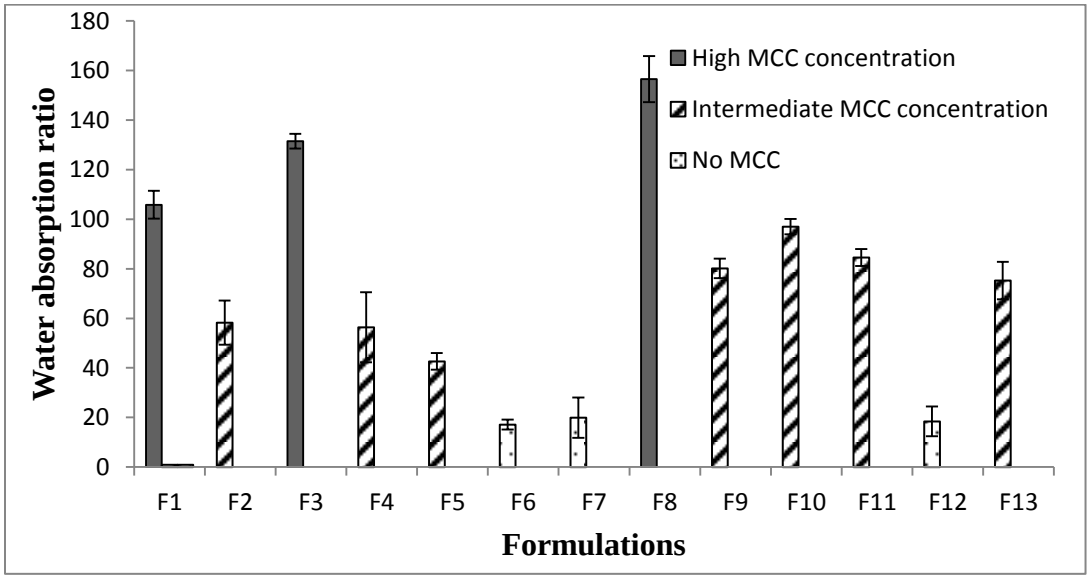


Figure 3.13: Water absorption ratio of DXM HBr-2-HPßCD ODTs

3.4.5.1 Mathematical modelling of data obtained from experimental design

Thirteen experiments were conducted to optimize the concentration of croscarmellose sodium (X_1) and microcrystalline cellulose (X_2) on the tablet hardness (Y_1), disintegration time (Y_2) and mean dissolution time (Y_3). Data for the CCD experimental runs are presented in *Table 3.9*. The measured responses are illustrated in *Figure 3.11 (a-c)* and shows that a selected formulation factor has a strong influence on the selected responses.

Table 3.9: Results of face centered CCD Experiments

Formulation No.	Croscarmellose Sodium-CCS (mg)	Microcrystalline Cellulose-MCC (mg)	Tablet Hardness-Y_1 (kp ± SD)	Disintegration Time- Y2 (sec ± SD)	MDT-Y3 (min)
F1	0	120	4.02 ± 0.48	12.83 ± 1.38	3.17
F2	0	60	8.15 ± 0.73	106.2 ± 5.80	6.06
F3	6	120	3.73 ± 0.35	9.17 ± 0.17	1.52
F4	6	60	7.05 ± 0.24	29.35 ± 4.16	1.40
F5	6	60	7.15 ± 1.04	25.90 ± 0.51	1.27
F6	6	0	6.76 ± 0.38	132.7 ± 4.47	4.17
F7	12	0	10.37 ± 0.92	140.7 ± 3.14	4.54
F8	12	120	1.51 ± 0.25	6.30 ± 0.69	1.20
F9	6	60	2.22 ± 0.13	11.40 ± 0.54	1.36
F10	12	60	2.07 ± 0.13	9.80 ± 0.39	1.30
F11	6	60	2.41 ± 0.24	13.65 ± 0.59	1.28
F12	0	0	10.87 ± 0.31	230.0 ± 5.97	5.78
F13	6	60	4.18 ± 0.20	14.80 ± 1.83	1.30

SD - Standard Deviation

ANOVA was applied to determine the significance level and magnitude of the effects of the process variables (factors) and interaction between the variables. The results confirm the adequacy of the model ($P < 0.05$) as shown in *Table 3.10*. The model identified the significant factors (X_1 and X_2) that affect the responses (Y_1, Y_2 and Y_3) of DXM HBr ODTs. In hardness of ODTs, concentration of microcrystalline cellulose (MCC) was significant; however the concentration of croscarmellose sodium (CCS) was not significant. In disintegration times and mean dissolution times for ODTs, the concentration of both CCS and MCC were significant. The interaction between the main variables (X_1X_2) was significant for disintegration time (Y_2); however the interaction between MCC and CCS were not significant for tablet hardness (Y_1) and MDT (Y_3).

Table 3.10: ANOVA for Tablet Hardness (Y_1), Disintegration Time (Y_2) and Mean Dissolution Time (Y_3)

Source	Sum of Squares			Degree of Freedom (df)			F value			p – value Prob > F		
	Y_1	**Y_2**	**Y_3**	**Y_1**	**Y_2**	**Y_3**	**Y_1**	**Y_2**	**Y_3**	**Y_1**	**Y_2**	**Y_3**
Model	72.37	61261.32	36.65	2	7	5	7.40	119.30	10.32	0.0107*	< 0.0001*	*0.0040**
X_1 - CSS	*13.78*	*4651.94*	*10.55*	*1*	*1*	*1*	*2.82*	*63.41*	*14.86*	*0.1242*	*0.0005**	*0.0063**
X_2 - MCC	*58.58*	*7640.13*	*12.35*	*1*	*1*	*1*	*11.98*	*104.15*	*17.39*	*0.0061**	*0.0002**	*0.0042**
X_1X_2	-	*1711.06*	*0.13*	-	*1*	*1*	-	*23.32*	*0.18*	-	*0.0048**	*0.6834*
X_1^2	-	*3002.52*	*7.26*	-	*1*	*1*	-	*40.93*	*10.22*	-	*0.0014**	*0.0151**
X_2^2	-	*5822.99*	*1.70*	-	*1*	*1*	-	*79.38*	*2.39*	-	*0.0003**	*0.1658*
$X_1^2X_2$	-	*908.57*	-	-	*1*	-	-	*12.39*	-	-	*0.0169**	-
$X_1X_2^2$	-	*785.86*	-	-	*1*	-	-	*10.71*	-	-	*0.0221**	-
Residual	48.92	366.79	4.97	10	5	7	-	-	-	-	-	-
Lack of Fit	*25.78*	*107.99*	*4.96*	*6*	*1*	*3*	*0.74*	*1.67*	*543.56*	*0.6459*	*0.2660*	< *0.0001**
Pure Error	*23.14*	*258.80*	*0.012*	*4*	*4*	*4*	-	-	-	-	-	-
Cor total	121.29	61628.11	41.62	12	12	12	-	-	-	-	-	-

* represents significant model terms with P-value < 0.05

The final mathematical models obtained from the design for the analysis of each response variable are as follows:

$$Y_1 = 5.43 - 1.52\,X_1 - 3.12\,X_2 \quad \text{(Eqn. 3.6)}$$

$$Y_2 = 20.75 - 48.23\,X_1 - 61.81\,X_2 + 20.68\,X_1X_2 + 32.97\,{X_1}^2 + 45.92\,{X_2}^2 - 26.10\,{X_1}^2X_2 + 24.28\,X_1{X_2}^2 \quad \text{(Eqn. 3.7)}$$

$$Y_3 = 1.53 - 1.33\,X_1 - 1.43\,X_2 - 0.18\,X_1X_2 + 1.62\,{X_1}^2 + 0.78\,{X_2}^2 \quad \text{(Eqn. 3.}$$

The above equations were derived by the best fit method and were used to make predictions about the response for given levels of each factor. Equations 3.6, 3.7 and 3.8 were used to describe the main effect of factors (X_1 and X_2) and their interaction (X_1X_2) on the responses (Y_1, Y_2 and Y_3). The coefficient values (regression coefficients) of X_1 and X_2 determine the effect of these variables on the response. Coefficients with more than one factor in the above equation represented either an interaction effect between both factors (X_1X_2) or quadratic relationships between factors indicated by higher order terms (X_1^2, X_2^2, $X_1^2X_2$ and $X_1X_2^2$). The relative impacts of the factors can be determined by comparing the factor coefficients. A positive sign indicates the synergistic effect while a negative sign reflects the antagonistic effect.

Equation 3.6 illustrates that both variables X_1 and X_2 have an antagonistic effects on tablet hardness (Y_1). Tablet hardness decreases with an increase in concentration of CSS and MCC. The antagonistic effect of CCS was not significant ($p > 0.05$) whereas the antagonistic effect of MCC was significant ($p < 0.05$) *(Table 3.10).*

Equation 3.7 illustrates that both variable X_1 and X_2 have significant ($p < 0.05$) antagonistic effects on the disintegration time (Y_2) with significant ($p < 0.05$) synergistic interaction effect. Disintegration time decreases with an increase in concentration of CCS and MCC, however it increases with an increase in the interaction between CCS and MCC. The equation also shows significant ($p < 0.05$) synergistic quadratic effects (X_1^2, X_2^2, $X_1X_2^2$) and significant ($p < 0.05$) antagonistic quadratic effects ($X_1^2X_2$). The quadratic effects are reflected by the curvature in the response surface and contour plot.

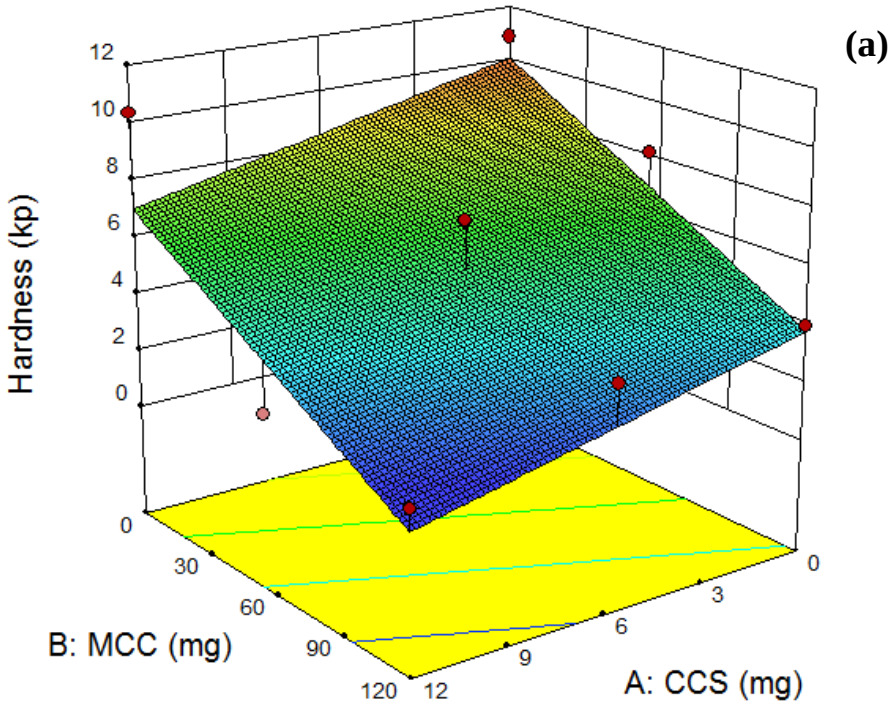
Equation 3.8 illustrates that both variable X_1 and X_2 have significant ($p < 0.05$) antagonistic effects on the mean dissolution time (MDT) with an insignificant ($p > 0.05$) antagonistic

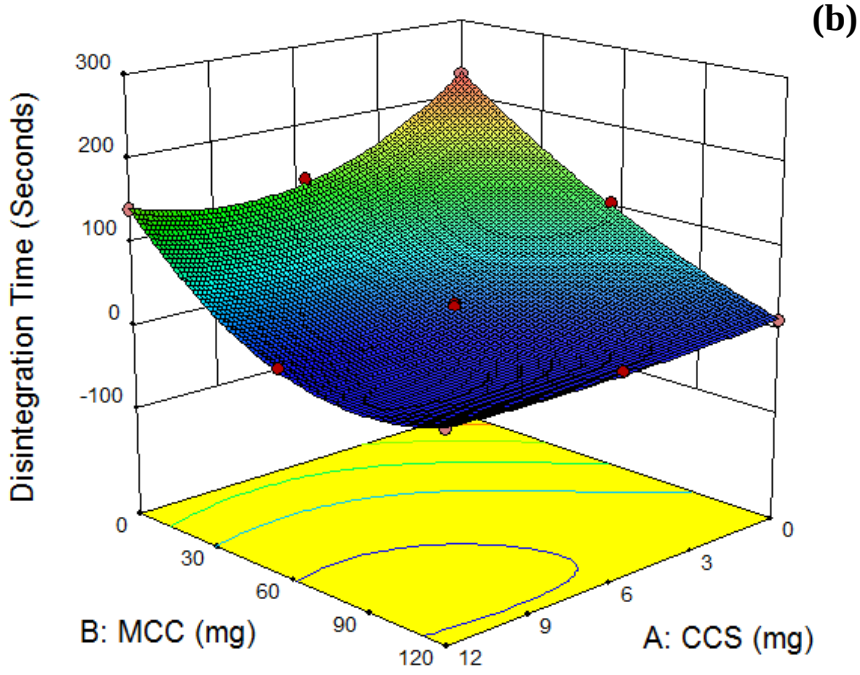
interaction effect. MDT decreases with an increase in concentration of CCS and MCC and their interaction. Equation 3.8 also shows significant ($p < 0.05$) synergistic quadratic effect (X_1^2) and an insignificant ($p > 0.05$) synergistic quadratic effect (X_2^2) indicated by the curvature in response surface and contour plot.

The antagonistic effects of X_1 and X_2 were minor with respect to tablet hardness (Y_1) and mean dissolution time (MDT) (Y_3) as compared to disintegration time (Y_2). The interaction effect between X_1 and X_2 and quadratic effects were minor with respect to (MDT) (Y_3) as compared to disintegration time (Y_2).

3.4.5.2. Analysis of the fitted data

The contour plots and 3D-response surface plots explained the effects of CSS and MCC concentration on tablet hardness, disintegration time and mean dissolution time of DXM HBr ODTs. *Figures 3.14a* and *3.15a* presents the effects of CSS and MCC concentration on tablet hardness. It can be seen that an increase in concentration of both CCS and MCC resulted in a decrease in tablet hardness. The optimum range of hardness was obtained at a decreased CSS concentration and an increased MCC concentration. *Figures 3.14b* and *3.15b* present the effects of CSS and MCC concentrations on disintegration time. It can be seen that an increase in concentrations of CSS, in the absence of MCC, shows the quadratic effect (i.e. disintegration time of the tablet decreases to reach its minimum at 6 mg of CCS and then starts increasing). The optimum disintegration time was obtained at a decreased CCS concentrations and mid-range concentrations of MCC. Similarly, an optimum mean dissolution time was observed at mid to high level concentrations of both CCS and MCC as presented in *Figures 3.14c* and *3.15c*.


(a)
Hardness (kp)
12
10
8
6
4
2
0
0
30
60
90
120
B: MCC (mg)
12
9
6
3
0
A: CCS (mg)


(b)
Disintegration Time (Seconds)
300
200
100
0
-100
0
30
60
90
120
B: MCC (mg)
12
9
6
3
0
A: CCS (mg)

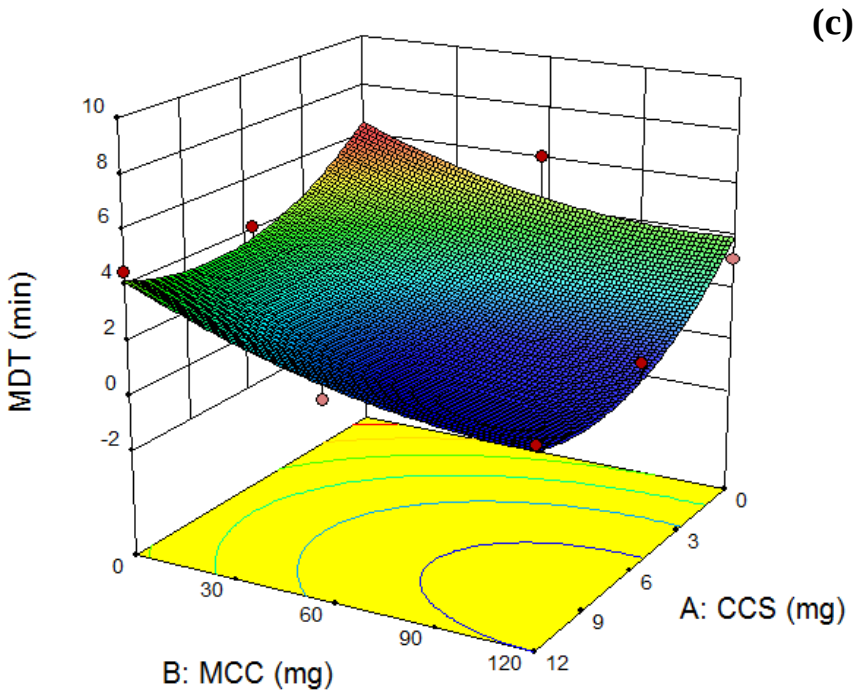
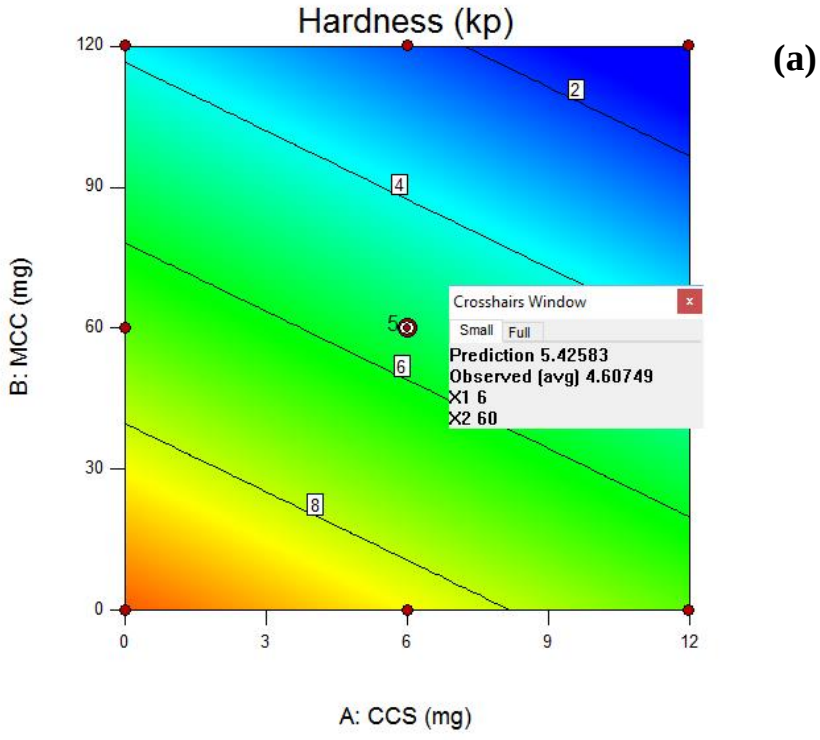
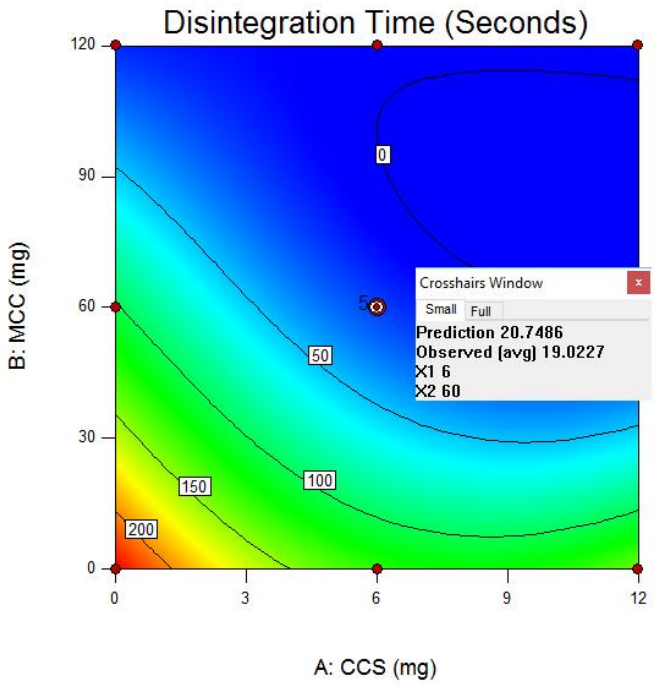
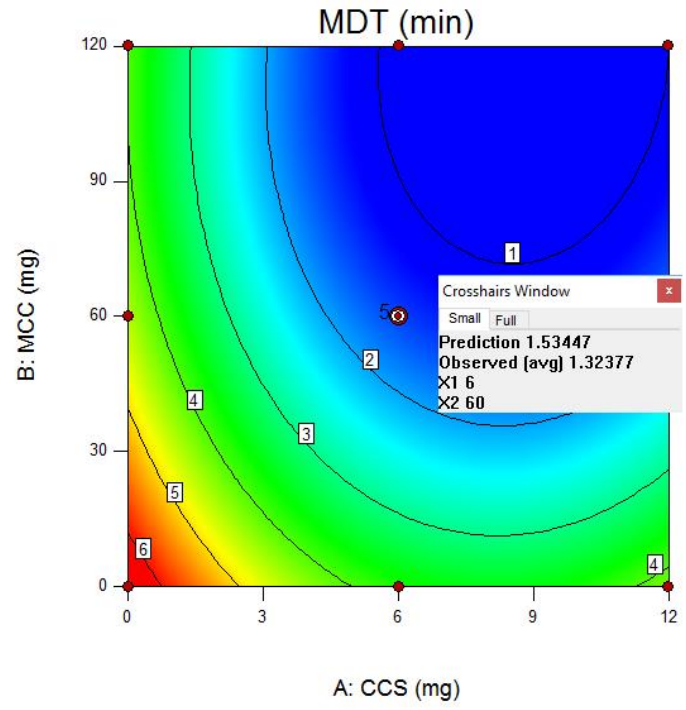


Figure 3.14: Response surface plot showing effect of CCS and MCC concentration on tablet hardness (a), disintegration time (b) and mean dissolution time (c)





(b)



(c)

Figure 3.15: Contour plot showing effect of CCS and MCC concentration on tablet hardness (a), disintegration time (b) and mean dissolution time (c)

3.4.5.3. Dissolution rate from ODTs

Figure 3.16 shows the *in-vitro* dissolution profiles of the DXM HBr ODT formulations. The mean dissolution time (MDT) for the formulations varied from 1.2 to 6.06 minutes (*Table 3.9*). Formulations F3, F4, F5, F8, F9, F10, F11, and F13 (with mid to high level concentrations of both CSS and MCC) showed MDTs in the range of 1.2 to 1.52 minutes. F1 and F2, in the absence of CCS, showed MDTs of 3.17 and 6.06 minutes, respectively. The decrease in MDT in the presence of CCS can be attributed to properties of the super disintegrants in rapidly disintegrating the tablets and facilitating dissolution [145]. Between formulation F1 and F2, higher concentrations of MCC decreased the MDTs. F6 and F7, in the absence of MCC, showed higher MDTs of 4.17 and 4.54 minutes, respectively. MCC is used as a diluent to ease the tablet compaction. However in addition to its dry binding properties, MCC is self-disintegrating with a low lubricant requirement due to its extremely low coefficient of friction and its very low residual die wall pressure and serves as a disintegrant as well [144]. This may be the reason that higher concentrations of MCC enhance tablet disintegration and improves dissolution. Similarly, F12 (in the absence of both CCS and MCC) showed a higher MDT of 5.78 minutes. Formulations with a higher concentration of mannitol (F6, F7, and F12) decreased the dissolution rates of the drug from the tablets since mannitol increases tablet hardness and compactability.

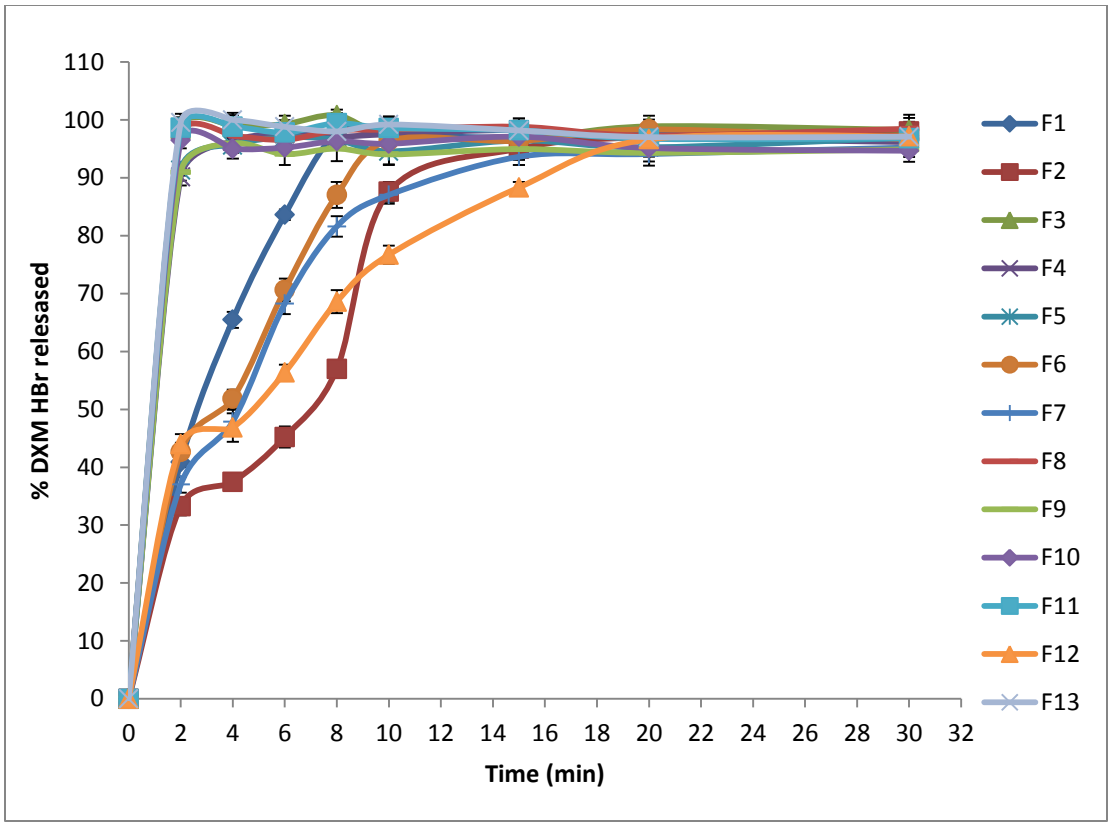


Figure 3.16: Dissolution profile of DXM HBr ODTs formulations

3.4.6. Validation of model and Optimization of process variables for DXM HBr ODTs

Validation of the regression equation or models was done by setting the checkpoint of CSS-X_1 and MCC-X_2 at 6mg and 60 mg, respectively (*Crosshair window, Figure 3.13a-c*). The values predicted by the model for tablet hardness (Y_1), disintegration time (Y_2) and MDT (Y_3) for the ODTs at the given checkpoint were in close agreement with the values observed which is illustrated in *Figure 3.17*.

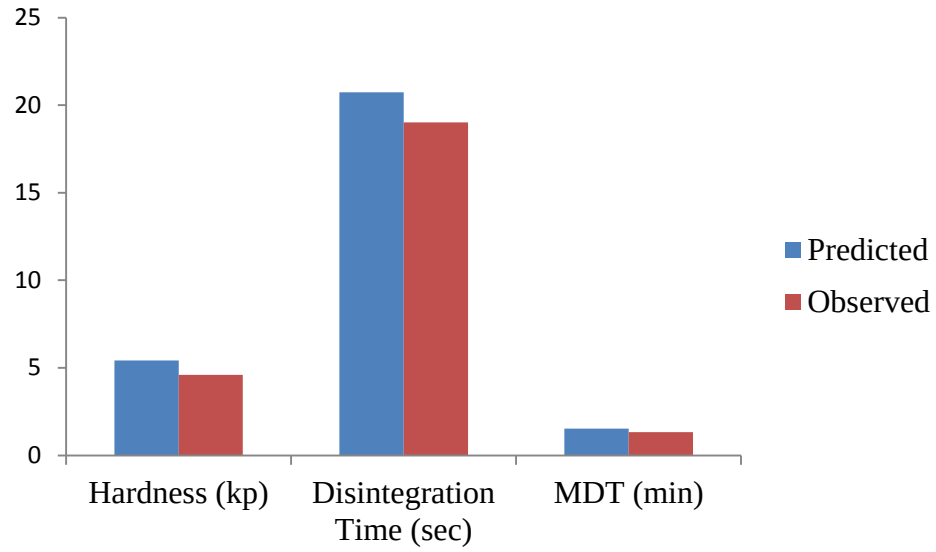


Figure 3.17: Comparison of predicted values from the model and observed values for ODTs

Numerical optimization was done using Design EXPERT *Version 10.0* software. The parameters for optimization presented in *Table 3.11* were chosen based on the analysis and results obtained for the response surface plots and contour plots.

Table 3.11: Parameters for Numerical Optimization

Constraints			
Name	**Goal**	**Lower Limit**	**Upper Limit**
X1 :CCS	is in range	0	12
X2 :MCC	is in range	0	120
Hardness	is target = 4.5	3.5	5.5
Disintegration Time	is target = 10	6.3	45
MDT	minimize	1.20822	6.06005

Figure 3.18 is an overlay plot with the design space (yellow area) for obtaining cyclodextrin based DXM HBr ODTs formulations.

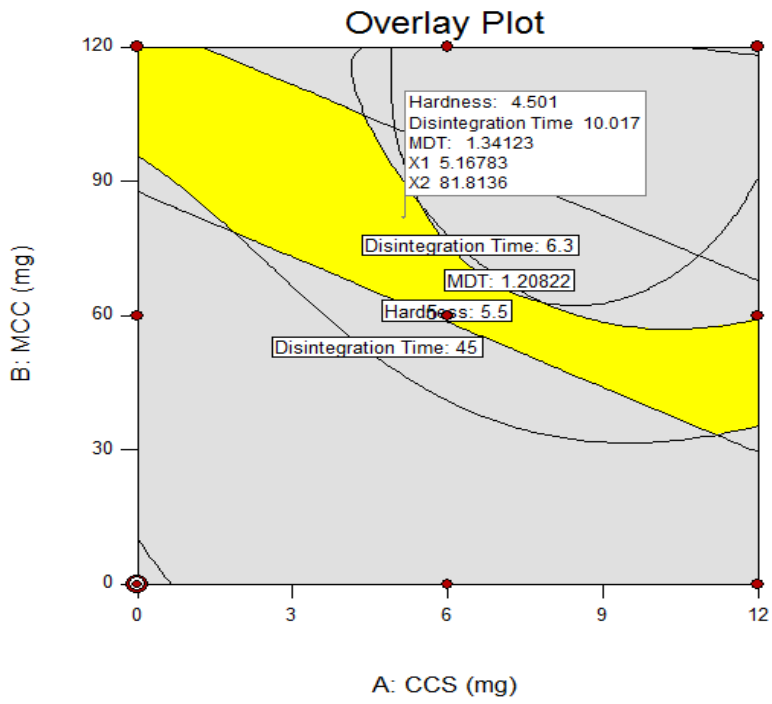


Figure 3.18: Overlay plot for the optimization parameters with design space (yellow area) to obtain DXM HBr ODTs

Table 3.12 shows the concentration of CCS and MCC, and properties for the optimum batches that the model predicted for achieving above goal set (*Table 3.11*). The optimum batch was selected based on the highest desirability of achieving the targeted or desired responses (i.e. tablet hardness, disintegration time, MDT) (*Table 3.12*).

Table 3.12: Properties of the optimized batches predicted by the model

Optimized batch	CCS	MCC	Hardness	Disintegration Time	MDT	Desirability	
1	5.168	81.814	4.500	10.000	1.341	0.991	Selected
2	10.869	54.161	4.500	10.000	1.687	0.966	
3	0.000	119.912	3.821	15.090	4.010	0.488	

Optimized batch yield results within the design space and thus confirmed the reliability of the numerical optimization process. Response surface plots showing the desirability of the CCS and MCC concentrations in the design space is shown in *Figure 3.19*.

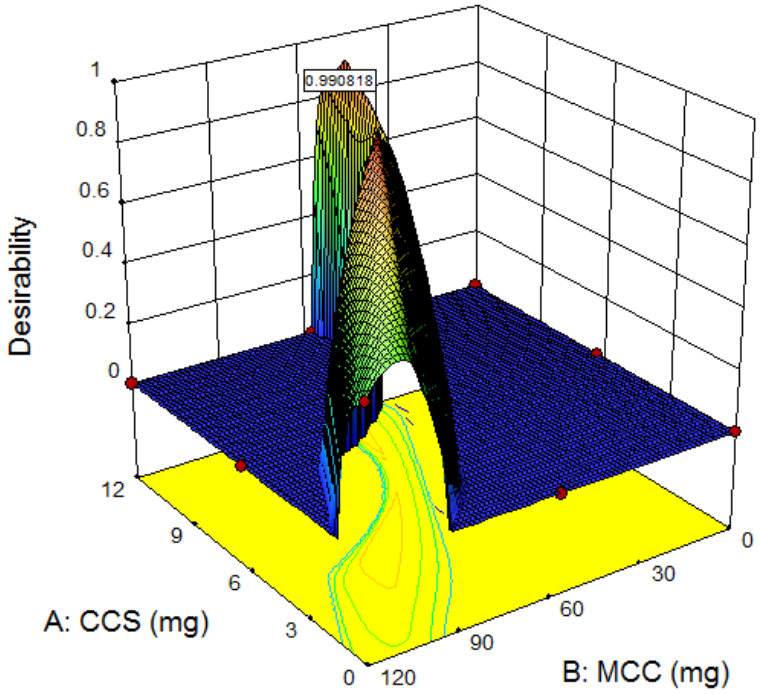


Figure 3.19: Response surface plot for CCS and MCC concentration desirability in design space

The optimum conditions for the ODT formulations predicted by the model are 5.168 mg (2.5%) of CCS and 81.814 mg (40%) of MCC (*Table 3.12*) to obtain the target hardness of 4.5 kp (between the range of 3.5to 5.5 kp hardness), target disintegration time of 10 seconds (between the range of 6.3 to 45 seconds) and minimum mean dissolution time (MDT) of 1.341 minutes. *Table 3.13* shows the optimized formula for 2-HPßCD inclusion complex based ODTs.

Table 3.13: Optimized formula for 2-HPßCD inclusion complex based DXM HBr ODTs

Ingredients	Weight[a] (mg)
DXM HBr-2-HPßCD inclusion complex (Lyophilized powder)	10*
Croscarmellose Sodium (CCS)	5.16 mg (2.5%)
Microcrystalline Cellulose (MCC)	81.81 mg (40%)
Sodium stearyl fumarate	2
Orange flavor	2
Mannitol	q.s. 200

[a]Total tablet weight = 200 mg

* Inclusion complex powder equivalent to 10 mg of DXM HBr

3.5. CONCLUSIONS

In the present study, we developed a novel ODT formulation for Dextromethorphan HBr. Dextromethorphan HBr is a cough suppressant and its formulation is widely used in pediatrics to relieve cough. While targeting the pediatric population, it is necessary to mask the bitter taste of the drug and develop a formulation suitable for children. The lyophilized inclusion complex of Dextromethorphan HBr with 2- hydroxyl propyl beta cyclodextrin enhanced the dissolution rate and showed the ability to mask the bitter taste of the drug which is vital for preparation of orally disintegrating tablets for pediatric use. The taste masking ability and mechanism of inclusion complex was proposed based on the characterization of the lyophilized inclusion complex powder using NMR spectroscopy and 2D-NMR ROESY as ultimate characterization tool. DXM HBr ODTs were successfully prepared using the DXM HBr-2HPßCD lyophilized complex powder. The ODTs were prepared using the direct compression method. There are several factors that affect the properties of DXM HBr ODTs prepared from inclusion complex. Concentration of the diluent and superdisntegrant are among the major factors affecting the ODTs properties. The composition of the diluent (MCC) and superdisintegrant (CSS) could be

optimized using response surface methodology with central composite design so as to obtain rapid dissolution and disintegration with acceptable tablet hardness and friability. This could enhance the drug absorption of ODTs from the oral mucosa producing a rapid relief from cough, ultimately resulting in improved patient adherence and convenience.

3.6. ACKNOWLEDGEMENTS

The authors would like to acknowledge Dr. Pannee Burckel, Chemical Instrumentation Scientist at University of Toledo for her help with Scanning Electron Microscopy and X-ray diffraction studies. We would like to thank Roquette America Inc. for providing samples of KLEPTOSE HPB (parenteral grade) used in this research. We would also like to thank JRS Pharma for providing samples of the tablet excipients.

References

1. Mennella, J.A. and G.K. Beauchamp, *Optimizing oral medications for children.* Clin Ther, 2008. **30**(11): p. 2120-32.
2. Mennella, J.A., et al., *The bad taste of medicines: overview of basic research on bitter taste.* Clin Ther, 2013. **35**(8): p. 1225-46.
3. Ivanovska, V., et al., *Pediatric drug formulations: a review of challenges and progress.* Pediatrics, 2014. **134**(2): p. 361-72.
4. Ernest, T.B., et al., *Developing paediatric medicines: identifying the needs and recognizing the challenges.* J Pharm Pharmacol, 2007. **59**(8): p. 1043-55.
5. Nunn, T. and J. Williams, *Formulation of medicines for children.* Br J Clin Pharmacol, 2005. **59**(6): p. 674-6.
6. Glendinning, J.I., *Is the bitter rejection response always adaptive?* Physiol Behav, 1994. **56**(6): p. 1217-27.
7. Meyerhof, W., et al., *Human bitter taste perception.* Chem Senses, 2005. **30 Suppl 1**: p. i14-5.
8. Mennella, J.A., M.Y. Pepino, and G.K. Beauchamp, *Modification of bitter taste in children.* Dev Psychobiol, 2003. **43**(2): p. 120-7.
9. Khan, M.A., *challanges in development of pediatric formulatiuons*. 2012, US-FDA.
10. Pawar, S. and A. Kumar, *Issues in the formulation of drugs for oral use in children: role of excipients.* Paediatr Drugs, 2002. **4**(6): p. 371-9.

11. Schirm, E., et al., *Lack of appropriate formulations of medicines for children in the community.* Acta Paediatr, 2003. **92**(12): p. 1486-9.

12. Roberts, R., et al., *Pediatric drug labeling: improving the safety and efficacy of pediatric therapies.* Jama, 2003. **290**(7): p. 905-11.

13. Butchko, H.H. and F.N. Kotsonis, *Acceptable daily intake vs actual intake: the aspartame example.* J Am Coll Nutr, 1991. **10**(3): p. 258-66.

14. Guidotti, J.L., *Laxative components of a generic drug.* Lancet, 1996. **347**(9001): p. 621.

15. Gershanik, J., et al., *The gasping syndrome and benzyl alcohol poisoning.* N Engl J Med, 1982. **307**(22): p. 1384-8.

16. McCloskey, S.E., et al., *Toxicity of benzyl alcohol in adult and neonatal mice.* J Pharm Sci, 1986. **75**(7): p. 702-5.

17. Madhusudhan Malladi, R.J., Radhmi Nair, Sanjay Wagh, Hari Shanjer Padakanti, Ashok Mateti, *Design and evaluation of tasted masked dextromethorphan hydrobromide oral disintegrating tablets.* Acta Pharm, 2010. **60**: p. 267-280.

18. Shaikh Sana, A.R., Nadkar Sumedha and Bharati Mahesh, *Formulation and Evaluation of taste masked oral suspension of Dextromethorphan Hydrobromide.* International Journal of Drug Development and Research, 2012. **4**(2): p. 159-172.

19. Samprasit, W., et al., *Preparation and evaluation of taste-masked dextromethorphan oral disintegrating tablet.* Pharm Dev Technol, 2012. **17**(3): p. 315-20.

20. (WHO), W.H.O. *Dextromethorphan Pre-Review report*. in *Expert Committe on Drug Dependence (ECDD)*. 2012. Hammamet, Tunisia.

21. Bem, J.L. and R. Peck, *Dextromethorphan. An overview of safety issues.* Drug Saf, 1992. **7**(3): p. 190-9.

22. Admisnistration, U.-F.a.D., *FDA warns against Abuse of Dextromethorphan (DXM)*, in *FDA Talk Paper*. 2005, US- Food and Drug Administration (FDA).

23. C, S.S., *Formulation and Evaluation of Dextromethorphan Hydrobromide Controlled Release Hollow Microspheres Using Natural Polymer.* Indonesian Journal of Pharmacy, 2014. **25**(3): p. 181.

24. Karaman, R., *Prodrugs for Masking the Bitter Taste of Drugs.* 2014.

25. Laza-Knoerr, A., R. Gref, and P. Couvreur, *Cyclodextrins for drug delivery.* Journal of drug targeting, 2010. **18**(9): p. 645-656.

26. Chaudhary, V. and J. Patel, *Cyclodextrin inclusion complex to enhance solubility of poorly water soluble drugs: A review.* International Journal of Pharmaceutical Sciences and Research, 2013. **4**(1): p. 68.

27. Bender, M.L. and M. Komiyama, *Cyclodextrin chemistry*. Vol. 6. 2012: Springer Science & Business Media.

28. Challa, R., et al., *Cyclodextrins in drug delivery: an updated review.* Aaps Pharmscitech, 2005. **6**(2): p. E329-E357.

29. Del Valle, E.M., *Cyclodextrins and their uses: a review*. Process biochemistry, 2004. **39**(9): p. 1033-1046.

30. Szejtli, J., *Introduction and general overview of cyclodextrin chemistry.* Chemical reviews, 1998. **98**(5): p. 1743-1754.

31. Uekama, K., F. Hirayama, and H. Arima, *Recent aspect of cyclodextrin-based drug delivery system.* Journal of inclusion phenomena and macrocyclic chemistry, 2006. **56**(1-2): p. 3-8.

32. Brewster, M.E. and T. Loftsson, *Cyclodextrins as pharmaceutical solubilizers.* Advanced drug delivery reviews, 2007. **59**(7): p. 645-666.

33. Szejtli, J., *Cyclodextrins and their inclusion complexes*. 1982: Akademiai Kiado.

34. Szejtli, J., *Past, present and futute of cyclodextrin research.* Pure and Applied Chemistry, 2004. **76**(10): p. 1825-1845.

35. Loftsson, T. and M.E. Brewster, *Pharmaceutical applications of cyclodextrins: basic science and product development.* J Pharm Pharmacol, 2010. **62**(11): p. 1607-21.

36. Rajewski, R.A. and V.J. Stella, *Pharmaceutical applications of cyclodextrins. 2. In vivo drug delivery.* Journal of pharmaceutical sciences, 1996. **85**(11): p. 1142-1169.

37. Frömming, K.-H. and J. Szejtli, *Pharmacokinetics and toxicology of cyclodextrins*, in *Cyclodextrins in Pharmacy*. 1994, Springer. p. 33-44.

38. Gould, S. and R.C. Scott, *2-Hydroxypropyl-β-cyclodextrin (HP-β-CD): a toxicology review.* Food and Chemical Toxicology, 2005. **43**(10): p. 1451-1459.

39. Loftsson, T. and M. Masson, *Cyclodextrins in topical drug formulations: theory and practice.* International Journal of Pharmaceutics, 2001. **225**(1): p. 15-30.

40. Yaksh, T.L., et al., *The utility of 2-hydroxypropyl-β-cyclodextrin as a vehicle for the intracerebral and intrathecal administration of drugs.* Life sciences, 1991. **48**(7): p. 623-633.

41. MAJEED, M.S.C., et al., *Process for preparing water soluble diterpenes and their applications*. 2012, Google Patents.

42. Cramer, F., W. Saenger, and H.-C. Spatz, *Inclusion compounds. XIX. 1a The formation of inclusion compounds of α-cyclodextrin in aqueous solutions. Thermodynamics and kinetics.* Journal of the American Chemical Society, 1967. **89**(1): p. 14-20.

43. Bergeron, R.J., M.A. Channing, and K.A. McGovern, *Dependence of cycloamylose-substrate binding on charge.* Journal of the American Chemical Society, 1978. **100**(9): p. 2878-2883.

44. Buvári, A. and L. Barcza, *Complex formation of phenol, aniline, and their nitro derivatives with β-cyclodextrin.* Journal of the Chemical Society, Perkin Transactions 2, 1988(4): p. 543-545.

45. Higuchi, T. and A. Connors, *Phase-solubility techniques.* 1965.

46. Loftsson, T. and M.E. Brewster, *Cyclodextrins as functional excipients: methods to enhance complexation efficiency.* J Pharm Sci, 2012. **101**(9): p. 3019-32.

47. Giri, T.K., et al., *A novel and alternative approach to controlled release drug delivery system based on solid dispersion technique.* Bulletin of Faculty of Pharmacy, Cairo University, 2012. **50**(2): p. 147-159.

48. Tiwari, R., et al., *Extended release promethazine HCl using acrylic polymers by freeze-drying and spray-drying techniques: formulation considerations.* Brazilian Journal of Pharmaceutical Sciences, 2009. **45**(4): p. 829-840.

49. JS, P., et al., *INCLUSION COMPLEX SYSTEM; A NOVEL TECHNIQUE TO IMPROVE THE SOLUBILITY AND BIOAVAILABILITY OF POORLY SOLUBLE DRUGS: A REVIEW.*

50. Baghel, S., H. Cathcart, and N.J. O'Reilly, *Polymeric Amorphous Solid Dispersions: A Review of Amorphization, Crystallization, Stabilization, Solid-State Characterization, and Aqueous Solubilization of Biopharmaceutical Classification System Class II Drugs.* J Pharm Sci, 2016.

51. Singh, R., et al., *Characterization of cyclodextrin inclusion complexes—a review.*

52. Liu, L. and S. Zhu, *Preparation and characterization of inclusion complexes of prazosin hydrochloride with β-cyclodextrin and hydroxypropyl-β-cyclodextrin.* Journal of pharmaceutical and biomedical analysis, 2006. **40**(1): p. 122-127.

53. Rajagopalan, N., S.C. Chen, and W.-S. Chow, *A study of the inclusion complex of amphotericin-B with γ-cyclodextrin.* International journal of pharmaceutics, 1986. **29**(2): p. 161-168.

54. Koontz, J.L., et al., *Cyclodextrin inclusion complex formation and solid-state characterization of the natural antioxidants alpha-tocopherol and quercetin.* J Agric Food Chem, 2009. **57**(4): p. 1162-71.

55. Stojanov, M., R. Wimmer, and K.L. Larsen, *Study of the inclusion complexes formed between cetirizine and alpha-, beta-, and gamma-cyclodextrin and evaluation on their taste-masking properties.* J Pharm Sci, 2011. **100**(8): p. 3177-85.

56. Inoue, Y., *NMR Studies of the Structure and Properties of Cyclodextrins and Their Inclusion Complexes*, in *Annual Reports on NMR Spectroscopy*, G.A. Webb, Editor. 1993, Academic Press. p. 59-101.

57. Szejtli, J., *The cyclodextrins and their applications in biotechnology.* Carbohydrate Polymers, 1990. **12**(4): p. 375-392.

58. Tiwari, G., R. Tiwari, and A.K. Rai, *Cyclodextrins in delivery systems: Applications.* Journal of Pharmacy and Bioallied Sciences, 2010. **2**(2): p. 72.

59. Vyas, A., S. Saraf, and S. Saraf, *Cyclodextrin based novel drug delivery systems.* Journal of Inclusion Phenomena and Macrocyclic Chemistry, 2008. **62**(1-2): p. 23-42.

60. Szejtli, J. and L. Szente, *Elimination of bitter, disgusting tastes of drugs and foods by cyclodextrins.* European Journal of pharmaceutics and biopharmaceutics, 2005. **61**(3): p. 115-125.

61. Sohi, H., Y. Sultana, and R.K. Khar, *Taste masking technologies in oral pharmaceuticals: recent developments and approaches.* Drug development and industrial pharmacy, 2004. **30**(5): p. 429-448.

62. Tripathi, A., et al., *Taste masking: a novel approach for bitter and obnoxious drugs.* JPSBR, 2011. **1**(3): p. 36-142.

63. Karaman, R., *Prodrugs for Masking the Bitter Taste of Drugs.* Application of Nanotechnology in Drug Delivery, 2014: p. 399-445.

64. Jijo, A. and M. Flowerlet, *Taste masking of peadiatric formulation: a review on technologies, recent trends and regulatory aspects.* Int J Pharm Pharm Sci, 2014. **6**: p. 12-19.

65. Ann, H.J., et al., *Effects of cyclodextrin derivatives on bioavailability of ketoprofen.* Drug development and industrial pharmacy, 1997. **23**(4): p. 397-401.

66. Choi, H.-G., et al., *Terfenadine–β-Cyclodextrin Inclusion Complex with Antihistaminic Activity Enhancement.* Drug development and industrial pharmacy, 2001. **27**(8): p. 857-862.

67. Tuleu, C. and J. Breitkreutz, *Educational paper: formulation-related issues in pediatric clinical pharmacology.* European journal of pediatrics, 2013. **172**(6): p. 717-720.

68. Castillo, J., et al., *Preparation and characterization of albendazole β-cyclodextrin complexes.* Drug development and industrial pharmacy, 1999. **25**(12): p. 1241-1248.

69. Latrofa, A., et al., *Complexation of phenytoin with some hydrophilic cyclodextrins: effect on aqueous solubility, dissolution rate, and anticonvulsant activity in mice.* European journal of pharmaceutics and biopharmaceutics, 2001. **52**(1): p. 65-73.

70. KAUKONEN, A.M., H. LENNERNÄS, and J.P. MANNERMAA, *Water-soluble β-Cyclodextrins in Paediatric Oral Solutions of Spironolactone: Preclinical Evaluation of Spironolactone Bioavailability from Solutions of β-Cyclodextrin Derivatives in Rats.* Journal of pharmacy and pharmacology, 1998. **50**(6): p. 611-619.

71. Arima, H., et al., *Comparative studies of the enhancing effects of cyclodextrins on the solubility and oral bioavailability of tacrolimus in rats.* Journal of pharmaceutical sciences, 2001. **90**(6): p. 690-701.

72. Narshima Reddy, D., et al., *Evaluation of piroxicam oral and transdermal preparations.* INDIAN JOURNAL OF PHARMACEUTICAL SCIENCES, 1992. **54**: p. 152-152.

73. Choudhury, S. and K.F. Nelson, *Improvement of oral bioavailability of carbamazepine by inclusion in 2-hydroxypropyl-β-cyclodextrin.* International journal of pharmaceutics, 1992. **85**(1): p. 175-180.

74. Miyaji, T., et al., *Improvement of oral bioavailability of fenbufen by cyclodextrin complexations.* Acta pharmaceutica nordica, 1991. **4**(1): p. 17-22.

75. Nervetti, A., U. Ambanelli, and G. Ugolotti, *Assessment of gastric mucosal damage by a new inclusion complex of piroxicam with β-cyclodextrin: a functional study by a scintigraphic method.* J Drug Dev, 1991. **4**(Suppl. 1): p. 39-42.

76. Loftsson, T., et al., *Effects of cyclodextrins on drug delivery through biological membranes.* Journal of pharmaceutical sciences, 2007. **96**(10): p. 2532-2546.

77. Zuo, Z., et al., *Flutamide-hydroxypropyl-ß-cyclodextrin Complex: formulation, physical characterization, and absorption studies using the caco-2 in vitro model.* J. Pharm. Pharmaceut. Sci, 2000. **3**(2): p. 220-227.

78. Pitha, J., E.J. Anaissie, and K. Uekama, *γ-cyclodextrin: Testosterone complex suitable for sublingual administration.* Journal of pharmaceutical sciences, 1987. **76**(10): p. 788-790.

79. Badawy, S.I.F., M.M. Ghorab, and C.M. Adeyeye, *Bioavailability of danazol-hydroxypropyl-β-cylodextrin complex by different routes of administration.* International journal of pharmaceutics, 1996. **145**(1): p. 137-143.

80. Jain, A.C., B.J. Aungst, and M.C. Adeyeye, *Development and in vivo evaluation of buccal tablets prepared using danazol–sulfobutylether 7 β-cyclodextrin (SBE 7) complexes.* Journal of pharmaceutical sciences, 2002. **91**(7): p. 1659-1668.

81. YOSHIDA, A., et al., *Improvement of chemical instability of digitoxin in aqueous solution by complexation with. BETA.-cyclodextrin derivatives.* Chemical and pharmaceutical bulletin, 1988. **36**(10): p. 4075-4080.

82. Szejtli, J., *Utilization of cyclodextrins in industrial products and processes.* Journal of Materials Chemistry, 1997. **7**(4): p. 575-587.

83. Meltzer, E., M. Welch, and N. Ostrom, *Pill swallowing ability and training in children 6 to 11 years of age.* Clinical pediatrics, 2006. **45**(8): p. 725-733.

84. Battu, S.K., et al., *Formulation and evaluation of rapidly disintegrating fenoverine tablets: effect of superdisintegrants.* Drug development and industrial pharmacy, 2007. **33**(11): p. 1225-1232.

85. Best, B.M., et al., *Pharmacokinetics of lopinavir/ritonavir crushed versus whole tablets in children.* Journal of acquired immune deficiency syndromes (1999), 2011. **58**(4): p. 385.

86. Shah, R.B., et al., *Tablet splitting of a narrow therapeutic index drug: a case with levothyroxine sodium.* Aaps Pharmscitech, 2010. **11**(3): p. 1359-1367.

87. Cassio, A., et al., *Comparison between liquid and tablet formulations of levothyroxine in the initial treatment of congenital hypothyroidism.* The Journal of pediatrics, 2013. **162**(6): p. 1264-1269. e2.

88. Buck, M.L. and C. Health, *Alternative forms of oral drug delivery for pediatric patients.* Pediatr Pharmacol, 2013. **19**: p. 1-6.

89. Abay, F. and T. Ugurlu, *Orally disintegrating tablets: a short review.* Journal of Pharmaceutics & Drug Development, 2015. **3**(3): p. 1.

90. Breitkreutz, J. and J. Boos, *Paediatric and geriatric drug delivery.* Expert Opinion on Drug Delivery, 2007. **4**(1): p. 37-45.

91. Schiermeier, S. and P.C. Schmidt, *Fast dispersible ibuprofen tablets.* European journal of pharmaceutical sciences, 2002. **15**(3): p. 295-305.

92. Badgujar, B.P. and A.S. Mundada, *The technologies used for developing orally disintegrating tablets: a review.* Acta pharmaceutica, 2011. **61**(2): p. 117-139.

93. Bhowmik, D., et al., *Fast dissolving tablet: an overview.* Journal of chemical and pharmaceutical research, 2009. **1**(1): p. 163-177.

94. Sastry, S.V., J.R. Nyshadham, and J.A. Fix, *Recent technological advances in oral drug delivery - a review.* Pharm Sci Technolo Today, 2000. **3**(4): p. 138-145.

95. Yapar, E.A., *Orally disintegrating tablets: an overview.* Journal of Applied Pharmaceutical Science, 2014. **4**(2): p. 118-125.

96. Manivannan, R., *Oral disintegrating tablets: A future compaction.* Drug Invention Today, 2009. **1**(1): p. 61-65.

97. Khuri, A.I. and S. Mukhopadhyay, *Response surface methodology.* Wiley Interdisciplinary Reviews: Computational Statistics, 2010. **2**(2): p. 128-149.

98. Bezerra, M.A., et al., *Response surface methodology (RSM) as a tool for optimization in analytical chemistry.* Talanta, 2008. **76**(5): p. 965-977.

99. Baş, D. and İ.H. Boyacı, *Modeling and optimization I: Usability of response surface methodology.* Journal of Food Engineering, 2007. **78**(3): p. 836-845.

100. Myers, R.H., et al., *Response surface methodology: a retrospective and literature survey.* Journal of quality technology, 2004. **36**(1): p. 53.

101. Montgomery, D.C., et al., *The hierarchy principle in designed industrial experiments.* Quality and reliability engineering international, 2005. **21**(2): p. 197-201.

102. Wu, W., G. Cui, and B. Lu, *Optimization of multiple evariables: application of central composite design and overall desirability.* CHINESE PHARMACEUTICAL JOURNAL-BEIJING-, 2000. **35**(8): p. 530-532.

103. Aslan, N., *Application of response surface methodology and central composite rotatable design for modeling and optimization of a multi-gravity separator for chromite concentration.* Powder Technology, 2008. **185**(1): p. 80-86.

104. Myers, R.H., *Response surface methodology. Edwards brothers.* Ann Arbor, MI, 1976.

105. Myers, R.H., D.C. Montgomery, and C.M. Anderson-Cook, *Response surface methodology: process and product optimization using designed experiments*. 2016: John Wiley & Sons.

106. Ferreira, S.C., et al., *Box-Behnken design: An alternative for the optimization of analytical methods.* Analytica chimica acta, 2007. **597**(2): p. 179-186.

107. Iskandarani, B., et al., *Simultaneous optimization of capsule and tablet formulation using response surface methodology*. Drug development and industrial pharmacy, 1993. **19**(16): p. 2089-2101.

108. Cantor, S.L., M.A. Khan, and A. Gupta, *Development and optimization of taste-masked orally disintegrating tablets (ODTs) of clindamycin hydrochloride.* Drug development and industrial pharmacy, 2015. **41**(7): p. 1156-1164.

109. Rahman, Z., A. Siddiqui, and M.A. Khan, *Orally disintegrating tablet of novel salt of antiepileptic drug: formulation strategy and evaluation.* European Journal of Pharmaceutics and Biopharmaceutics, 2013. **85**(3): p. 1300-1309.

110. Ahmed, I.S. and M.H. Aboul-Einien, *In vitro and in vivo evaluation of a fast-disintegrating lyophilized dry emulsion tablet containing griseofulvin.* European journal of pharmaceutical sciences, 2007. **32**(1): p. 58-68.

111. Late, S.G., Y.-Y. Yu, and A.K. Banga, *Effects of disintegration-promoting agent, lubricants and moisture treatment on optimized fast disintegrating tablets.* International Journal of Pharmaceutics, 2009. **365**(1): p. 4-11.

112. Rauck, R.L., et al., *Efficacy and long-term tolerability of sublingual fentanyl orally disintegrating tablet in the treatment of breakthrough cancer pain.* Current medical research and opinion, 2009. **25**(12): p. 2877-2885.

113. Shangraw, R.F., *Compressed tablets by direct compression.* Pharmaceutical dosage forms: Tablets, 1989. **1**: p. 195-246.

114. Bandelin, F.J., *Compressed tablets by wet granulation.* Pharmaceutical dosage forms: tablets, 1989. **1**(2): p. 173-175.

115. Ringard, J. and A. Guyot-Hermann, *Calculation of disintecrant critical concentration in order to optimize tablets disintegration.* Drug Development and Industrial Pharmacy, 1988. **14**(15-17): p. 2321-2339.

116. Saenger, W., *Cyclodextrin inclusion compounds in research and industry.* Angewandte Chemie International Edition in English, 1980. **19**(5): p. 344-362.

117. Veiga, F., et al., *Inclusion complexation of tolbutamide with β-cyclodextrin and hydroxypropyl-β-cyclodextrin.* International Journal of Pharmaceutics, 1996. **129**(1): p. 63-71.

118. Ono, N., et al., *Reduction of bitterness of antihistaminic drugs by complexation with β-cyclodextrins.* Journal of pharmaceutical sciences, 2011. **100**(5): p. 1935-1943.

119. Waleczek, K., et al., *Phase solubility studies of pure (−)-α-bisabolol and camomile essential oil with β-cyclodextrin.* European Journal of Pharmaceutics and Biopharmaceutics, 2003. **55**(2): p. 247-251.

120. Figueiras, A., et al., *Solid-state characterization and dissolution profiles of the inclusion complexes of omeprazole with native and chemically modified β-cyclodextrin.* European Journal of Pharmaceutics and Biopharmaceutics, 2007. **67**(2): p. 531-539.

121. Samprasit, W., et al., *Preparation and evaluation of taste-masked dextromethorphan oral disintegrating tablet.* Pharmaceutical development and technology, 2012. **17**(3): p. 315-320.

122. Malladi, M., et al., *Design and evaluation of taste masked dextromethorphan hydrobromide oral disintegrating tablets.* Acta pharmaceutica, 2010. **60**(3): p. 267-280.

123. Singh, B., S.K. Chakkal, and N. Ahuja, *Formulation and optimization of controlled release mucoadhesive tablets of atenolol using response surface methodology.* AAPS PharmSciTech, 2006. **7**(1): p. E19-E28.

124. Muthuvelayudham, R. and T. Viruthagiri, *Application of central composite design based response surface methodology in parameter optimization and on cellulase production using agricultural waste.* International Journal of Chemical and Biological Engineering, 2010. **3**(2): p. 97-104.

125. Late, S.G. and A.K. Banga, *Response surface methodology to optimize novel fast disintegrating tablets using β cyclodextrin as diluent.* AAPS PharmSciTech, 2010. **11**(4): p. 1627-1635.

126. Marques, H.C., J. Hadgraft, and I. Kellaway, *Studies of cyclodextrin inclusion complexes. I. The salbutamol-cyclodextrin complex as studied by phase solubility and DSC.* International Journal of Pharmaceutics, 1990. **63**(3): p. 259-266.

127. Rahman, Z., A.S. Zidan, and M.A. Khan, *Risperidone solid dispersion for orally disintegrating tablet: its formulation design and non-destructive methods of evaluation.* International journal of pharmaceutics, 2010. **400**(1): p. 49-58.

128. Costa, P. and J.M.S. Lobo, *Modeling and comparison of dissolution profiles.* European journal of pharmaceutical sciences, 2001. **13**(2): p. 123-133.

129. Fernandes, C.M., M.T. Vieira, and F.J.B. Veiga, *Physicochemical characterization and in vitro dissolution behavior of nicardipine–cyclodextrins inclusion compounds.* European journal of pharmaceutical sciences, 2002. **15**(1): p. 79-88.

130. Williams III, R.O., V. Mahaguna, and M. Sriwongjanya, *Characterization of an inclusion complex of cholesterol and hydroxypropyl-β-cyclodextrin.* European journal of pharmaceutics and biopharmaceutics, 1998. **46**(3): p. 355-360.

131. Zingone, G. and F. Rubessa, *Preformulation study of the inclusion complex warfarin-β-cyclodextrin.* International journal of pharmaceutics, 2005. **291**(1): p. 3-10.

132. Giunta, A.A., S.F. Wojtkiewicz, and M.S. Eldred. *Overview of modern design of experiments methods for computational simulations*. in *Proceedings of the 41st AIAA aerospace sciences meeting and exhibit, AIAA-2003-0649*. 2003.

133. Neter, J., et al., *Applied linear statistical models*. Vol. 4. 1996: Irwin Chicago.

134. Bi, Y., et al., *Preparation and evaluation of a compressed tablet rapidly disintegrating in the oral cavity.* Chemical and pharmaceutical bulletin, 1996. **44**(11): p. 2121-2127.

135. Uekama, K., et al., *Inclusi on complexations of steroid hormones with cyclodextrins in water and in solid phase.* International Journal of Pharmaceutics, 1982. **10**(1): p. 1-15.

136. Jun, S.W., et al., *Preparation and characterization of simvastatin/hydroxypropyl-β-cyclodextrin inclusion complex using supercritical antisolvent (SAS) process.* European Journal of Pharmaceutics and Biopharmaceutics, 2007. **66**(3): p. 413-421.

137. Ruan, L.-P., et al., *Improving the solubility of ampelopsin by solid dispersions and inclusion complexes.* Journal of Pharmaceutical and Biomedical analysis, 2005. **38**(3): p. 457-464.

138. Coates, J., *Interpretation of infrared spectra, a practical approach.* Encyclopedia of analytical chemistry, 2000.

139. Hiremath, S., et al., *Dissolution enhancement of gliclazide by preparation of inclusion complexes with [beta]-cyclodextrin.* Asian journal of Pharmaceutics, 2008. **2**(1): p. 73.

140. Veiga, F.J.B., et al., *Molecular Modelling and 1H-NMR: Ultimate Tools for the Investigation of Tolbutamide:. BETA.-Cyclodextrin and Tolbutamide: Hydroxypropyl-. BETA.-Cyclodextrin Complexes.* Chemical and pharmaceutical bulletin, 2001. **49**(10): p. 1251-1256.

141. Ahmed, S., et al., *Comparative dissolution characteristics of bropirimine-β-cyclodextrin inclusion complex and its solid dispersion with PEG 6000.* International journal of pharmaceutics, 1993. **96**(1-3): p. 5-11.

142. Wang, S., Y. Ding, and Y. Yao, *Inclusion complexes of fluorofenidone with β-cyclodextrin and hydroxypropyl-β-cyclodextrin.* Drug development and industrial pharmacy, 2009. **35**(7): p. 808-813.

143. Badawy, S.I., et al., *Effect of spray-dried mannitol on the performance of microcrystalline cellulose-based wet granulated tablet formulation.* Pharmaceutical development and technology, 2010. **15**(4): p. 339-345.

144. Thoorens, G., et al., *Microcrystalline cellulose, a direct compression binder in a quality by design environment—A review.* International Journal of Pharmaceutics, 2014. **473**(1–2): p. 64-72.

145. Balasubramaniam, J. and T. Bee, *The influence of superdesintegrant choice on the rate of drug dissolution.* Pharm Technol, 2009. **21**: p. S1-S2.