PREPARATION AND CHARACTERIZATION OF ENSET STARCH CITRATE AS A DISINTEGRANT IN TABLETS

BY

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A THESIS SUBMITTED TO THE SCHOOL OF PHARMACY, DEPARTMENT OF PHARMACEUTICS AND SOCIAL PHARMACY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN PHARMACEUTICS

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Addis Ababa University School of Graduate Studies

This is to certify that the thesis undertaken by Gebregziabiher Weldegiorgis, entitled "*Preparation and Characterization of Enset Starch Citrate as a Disintegrant in Tablets*" and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Pharmaceutics complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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ABSTRACT

Maize, cassava, wheat and potato are the main botanical origins for starch production with only minor quantities of rice. Physical and chemical modifications have been used to change the granular structure of starch and modify their functional properties to eliminate some of the undesirable properties, making them more suitable for specific uses. As native starches are poor disintegrants, they have been modified to improve their swellability and hence their disintegration efficiency in matrix tablets.

The main objective of this study is to prepare enset starch citrate and investigate its disintegration capacity in tablet formulations.

In this study, enset and potato starches were modified by chemical method under dry reaction conditions. Starch citrates of both starches were obtained under heterogeneous conditions as a product of the reaction of starch and citric acid in the presence of sodium hydroxide at eight different reaction conditions in each starch. The influences of pH of medium, reaction temperature and moisture content on the reaction process were investigated by treating both starches under acidic conditions. The disintegration capacity of the enset starch citrate (ESC) was evaluated in ibuprofen tablets compressed by direct compression method. Ibuprofen, a practically insoluble drug was used as a model drug. The starch citrates (SCs) prepared were studied for their gelling property, swelling power and solubility, settling volume, morphology, degree of substitution (DS), hydration capacity and moisture sorption profiles. A three level factorial design with two replicates at the center was used to study the influences of compression force and ESC levels on disintegration time (DT), hardness and friability.

The results indicated that ESC is a potential disintegrant; SCs showed greater settling volume at neutral pH than in acidic medium; and ESC attained a swelling power of 1500% at 65 ^{0}C. The DS of the ESC selected for the formulation was 0.11 and with a hydration capacity of 6.43 g/g. The SCs had higher settling volume in distilled water (DW) than in acidic medium. The SCs prepared were more hygroscopic than the native starch. Ibuprofen and ESC compatibility study by Differential Scanning Colorimetry (DSC) indicated that there is no incompatibility between them. Scanning Electron Micrograph (SEM) images of the ESC revealed that the granule characteristic is completely different from that of the native enset starch (NES). The comparative study of ibuprofen tablets containing ESC and sodium starch

glycolate (SSG) as disintegrants also revealed the superiority of SSG although the ESC produced harder tablets than SSG. Ibuprofen tablets included in the study design revealed that the disintegration times (DT) of the tablets ranged between 25 and 187 sec.; hardness ranged from 62.7 to 151 N, while friability ranged from 0.09 to 0.17%. Simultaneous optimization of DT, hardness and friability provided values of 25 sec, 84.3 N and 0.14%, respectively at compression force of 5.15 KN and ESC concentration of 4% respectively as the optimum point. Dissolution study of the optimized formulation fulfilled USP requirements. The optimized formulation was validated and it was confirmed that the predicted and measured values were in agreement.

Therefore, it can be concluded that ESC can be used as an alternative disintegrant in insoluble drug tablet formulations.

Keywords: Enset starch, Enset starch citrate, Citric acid, Disintegrant, Optimization

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Table of Contents

List of Tables

List of Figures

Acronyms

Adj R^2:	Adjusted R-squared
ANOVA:	Analysis of variance
CF:	Compression force
DisC:	Disintegrant concentration
DoE:	Design of experiments
DS:	Degree of substitution
DSC:	Differential scanning calorimetry
DW:	Distilled Water
ED:	Esterification degree
ESC:	Enset starch citrate
ICH:	International conference on harmonization
LOF:	Lack of fit
MCC:	Microcrystalline cellulose
NES:	Native enset starch
Pred R^2:	Predicted R-squared
PRESS:	Predicted Residual Sum of Square
RH:	Relative humidity
RSM:	Response surface methodology
SC:	Starch citrate
SEM:	Scanning electron micrograph
SP:	Swelling power
SSF:	Simulated salivary fluid
SSG:	Sodium starch glycolate
USP/NF:	United States Pharmacopeia/National Formulary
WSI:	Water solubility index

1 INTRODUCTION

1.1 Starch

Starch is a widely available, naturally occurring carbohydrate reserve in plants, which generally exists in the form of minute granules ranging from 1 to 100 μm or more in diameter. It is an amorphous polymeric nature, white in appearance, and relatively tasteless and odourless. It is insoluble in cold water and organic solvents such as ethanol, ether, and acetone. Starch is hygroscopic in nature and absorbs water when equilibrated under normal atmospheric condition until the amount present is 10 – 17%. Starch is extracted from relevant plants by the simple combination of grinding of the starch rich plant part followed by wet separation techniques (Cornell, 2004; Odeku, 2013).

Starch is composed of two polymers called amylose and amylopectin, the relative amount of each varies depending on the source (Gebre-Mariam and Schmidt, 1996a). Amylose is a linear polymer with molecular weight of less than 0.5 million Dalton (degree of polymerization ranging from (5×10^2- 6×10^3) depending on its botanical source. Amylose macromolecules consist of D-glucopyranose units joined by α-1,4-acetal linkages. Amylopectin molecules are much larger and highly branched with molecular weight of 50 - 100 million Dalton and degree of polymerization of about $3 \times 10^5 – 3 \times 10^6$. The molecules contain α-1,4 linear bonds, and is branched through α -1,6 linkages (Nelson and Wang, 1977). The structure of amylose and amylopectin is given in Figure 1.1.

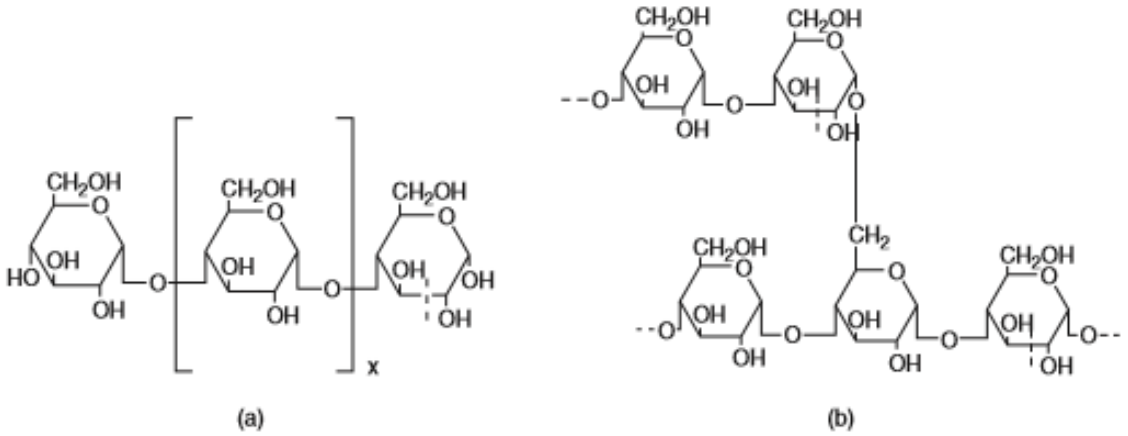

Figure 1.1: Schematic diagram of (a) amylose and (b) amylopectin

In 2012, the world production of starch was 75 million tons, of which more than half was produced in the United States (Waterschoot *et al.*, 2015); in 2010 the figure was 70 million tons (Spychaj *et al.,* 2013*).* Maize, cassava, wheat and potato are the main botanical origins for starch production with only minor quantities of rice and other starches being produced. These starches are either used by industry as such or following some conversion. When selecting and developing starches for specific purposes, it is important to consider the differences between starches of varying botanical origin. Differences in properties are largely defined by differences in amylose and amylopectin structures and contents, granular organization, presence of lipids, proteins and minerals and starch granule size (Waterschoot *et al.,* 2015).

Starch has wide industrial applications. It is one of the most widely used biomaterial in the food, textile, cosmetic, plastic, adhesives, paper and pharmaceutical industries. Starch is one of the most widely used excipients in the food and pharmaceutical industries where they are used as fillers, glidants, thickeners, binders, disintegrants as well as gelling, bulking, and water retention agents (Odeku, 2013). Due to its physicochemical nature, relative inertness, abundance and economy of price, starch is described as an excipient of choice and listed as one of the most popular eight tablet disintegrants that produce better disintegration (Table 1.1). Its high industrial demand requires that cheaper sources should continually be sought to meet the global needs (Mustapha *et al*., 2010).

Starch has been used as one of the best known excipients in various pharmaceutical formulations. This is due to the fact that native starch, in one hand, can provide essential tablet properties for drug release; on the other hand the presence of different structural features may be exploited for its modification to serve different functions. Rapid disintegrating tablet preparations and ability to add bulk to the drug product are two main factors provided by native starches. However due to poor powder flowability and to limitations in other properties, native starch has been chemically and/or physically modified. Consecutively a diversity of functional excipients are achieved by modification and/or processing of native starch, some of which perform as fillers, binders (Gebre-Mariam and Nikolayev, 1993; Gebre-Mariam and Schmidt, 1996a) and disintegrants, some as superdisintegrants (Gebre-Mariam *et al*., 1996), and others as controlled release matrices (Belachew and Gebre-Mariam, 2009; Rashid *et al.,* 2013)

1.2 Enset

Ensete ventricosum (Welw.) Cheeseman belongs to the family Musaceae, and the genus Ensete. It looks like a large, thick, single-stemmed banana plant. Both enset and banana have an underground corm, a bundle of leaf sheaths that form the pseudostem, and large leaves. Enset, however, is usually larger than banana, up to 10 m tall and pseudostem up to one meter in diameter. Because of its resemblance to the banana plant, enset is often referred as "false banana" as it does not bear edible fruit (Sirnrnonds, 1985, Wondimu *et al*., 2014). The crop has high yield and is highly drought resistant. It takes about 4 - 5 years for maturation at which time a single corm can give 40 kg of food (Hirose *et al.*, 2010).

E. ventricosum is an important traditional Ethiopian staple crop. It is widely distributed in eastern and southern Africa but only cultivated in southern and south western Ethiopia; it is concentrated in the southern high lands. It accumulates starch mainly in the pseudo-stem and underground corms which are utilized as a starch source for food and industrial purpose. Enset plant in the garden and its parts is shown in Figure 1.2.

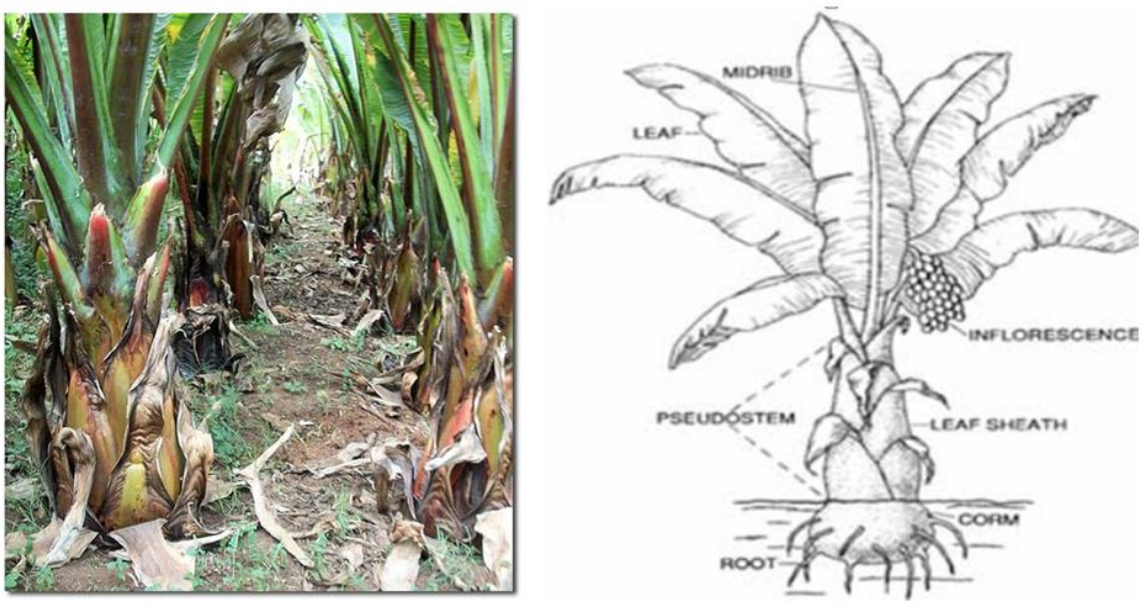

Figure 1.2: Enset plant in the field (A) and its parts (B).

E. ventricosum, hereinafter called as enset, is an important co-staple crop for greater than 20% of the Ethiopian population and, the edible parts are formed by the psydeo-stem and the underground corm. The major foods obtained from enset are kocho, bulla and amicho. Kocho is the bulk of the fermented starch obtained from the mixture of scraped leaf sheaths and

underground stem base. Bulla is the water soluble starchy product that may be separated from kocho during processing by squeezing and decanting the liquid. Amicho is the fleshy, inner portion of enset corm of usually a younger plant, which may be cooked and eaten separately, tasting similar to potato (Debebe *et al.*, 2006).

The proximate analysis of enset starch on dry weight basis (w/w) consists of 99.24% starch, 0.35% protein, o.16% ash, 0.25% fat, and it contains 14% moisture. Coulter counter analysis revealed the average granule size of the starch to be 37.7 µm which is comparable to that of potato starch (38.2 µm). Size distribution of the granules was reported to show a normal distribution curve between 15 and 60 µm, sharper than that of potato starch, which was broadly distributed from 5 to 65 µm. According to a study by Gebre-Mariam and Schmidt (1996a), the amylose content of enset starch was estimated to be 29.0%. Another study showed the amylose content to be 21% (Hirose *et al.*, 2010). Both studies showed the amylose content of enset starch and potato starch to be comparable. The variation could arise from differences in the methodologies used for determination of the amylose content. Enset starch exhibits typical X-ray diffraction (XRD) pattern of B-type with a distinctive maximum peak at around 17^0 2Ɵ. Its swelling power and solubility values are lower than potato starch but much higher than maize starch (Gebre-Mariam and Armstrong, 1995; Hirose *et al.*, 2010; Lertphanich *et al.*, 2013)

Lertphanich *et al*. (2013) showed that the final degree of enzyme hydrolysis of granular starches (after 180 min incubation) varied significantly between starches. Enset starch had the lowest values (2.3%). The enzyme hydrolysis kinetics curves of granular starches are shown elsewhere (Lertphanich *et al*. 2013). Among the starches: cassava, taro, yam bean, water caltrop, mungbean and chickpea, enset starch was least susceptible to α-amylase and glucoamylase hydrolysis at sub-gelatinization temperature (37 ^{0}C). The authors demonstrated that this could be related to granule size as the smaller granules have greater surface areas and therefore better enzyme accessibility. They showed enset starch granules are less susceptible to enzyme hydrolysis, among other factors, largely due to its large granule size (Lertphanich et al., 2013).

The disintegration and binding properties of enset starch have been evaluated in comparison to potato starch. The results showed that enset starch exhibited poorer disintegration property than potato starch. The authors concluded that although enset starch is a less effective

disintegrant than potato starch, it can be used as a disintegrant at optimum concentration suggesting that enset could be used as an alternative source of starch in tablet formulations (Gebre-Mariam and Nikolayev, 1993; Gebre-Mariam and Armstrong, 1995).

1.3 Starch Modification

Since time immemorable various attempts are being made in order to modify this highly flexible polymer with the aim to enhance the positive attributes and eliminate the short comings of the native starches. Modifications are generally made by physical methods like osmotic pressure treatment, deep freezing and thrashing; chemical methods that primarily include derivatization such as etherification, esterification and cross-linking, oxidation, cationization and grafting of starch; enzyme degradation techniques and genetic modification which involves the transgenic techniques targeting the various enzymes involved in starch biogenesis. All these techniques produce a variety of derivatives with altered phsico-chemical properties and modified structural attributes of high technological (Neelam *et al.*, 2012).

Physical and chemical modifications have been used to change the granular structure of starch and modify their functional properties to eliminate some of the undesirable properties, making them more suitable for specific uses. With the versatility of starches in various drug dosage forms, there is the need to continue to develop new starch excipients with suitable properties to meet the special needs of drug formulators (Odeku, 2013).

To obtain new properties for starch in order to overcome some of its shortcomings and make a more suitable alternative for particular applications, the starch can be modified. Three main types of modifications can be performed: reduction of chain length, pure substitutions and cross-linking reactions. Starch can be modified by substitution reactions on the three hydroxyl groups present on the anhydroglucose unit where the degree of substitution, DS, is the average number of hydroxyl groups in the anhydroglucose unit which have been substituted. The highest DS possible is thus 3. The functional properties of modified starches are dependent on both the chemical nature of the modifications and the DS (Wurzburg, 1986).

When the demand for faster disintegration and drug dissolution increased, more active agents were searched. At first, the swelling properties of native starches were improved by carboxymethylation, and the integrity of the starch grains were kept by cross-linking. The main products of this group are Primojel® and Explotab®. They have a high swelling capacity

so they are very effective even at low concentrations (Gebre-Mariam *et al.*, 1996). Starch silicon dioxide co-precipitate has also been demonstrated to act as a superdisintegrant (Nagpal *et al*., 2012). In other works, carboxymethylated starch slightly cross-linked with (sodium tripolyphosphate, citric acid, or epichlorohydrin) designated for tablet disintegrant was synthesized by extrusion process for short residence time (Spychaj *et al*., 2013).

Chemical modification involves the introduction of functional groups into the starch molecule, resulting in markedly altered physico-chemical properties. Such modification of native granular starches profoundly alters their gelatinization, pasting and retrogradation behavior. The chemical and functional properties achieved when modifying starch by chemical substitution depends on starch source, reaction condition, type of substituent, extent of substitution, and the distribution of the substituent in the starch molecule (Neelam *et al*., 2012).

1.3.1 Starch citrate

1.3.1.1 Reaction Pathways of the Preparation of Starch Citrate

Citric acid is an organic acid with three carboxyl groups and one hydroxyl group. Hence, it can react with the hydroxyl groups in starch to create esters, which can result in mono-, di- and tri-esters. The reaction is catalysed by increasing the pH or adding Lewis acids, as shown in Figure 1.3 and Figure 1.4. The reaction is shown to be promoted by high temperature treatment well above 100 °C (Wing and Peoria, 1996). Citric acid forms reactive anhydride up on heating by losing water molecule. Then the reactive anhydride can react with starch which is present in the reaction mixture to form starch citrate. The reaction involved in the preparation of the mono-ester is shown in Figure 1.3 (Fajd and Marton, 2004).

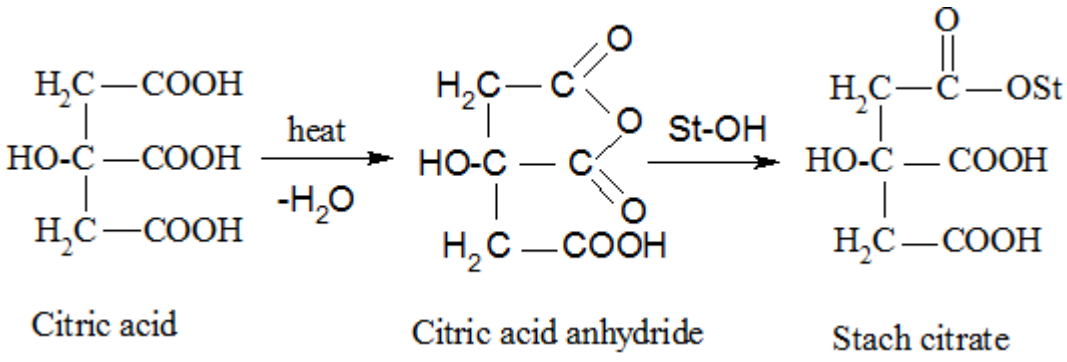

Figure 1.3: Thermochemical reaction of citric acid with starch resulting in citrate substituted starch (Fajd and Marton, 2004).

If heated beyond an optimal reaction time, starch citrate dehydrates again and cross-linking can take place (Figure 1.4).

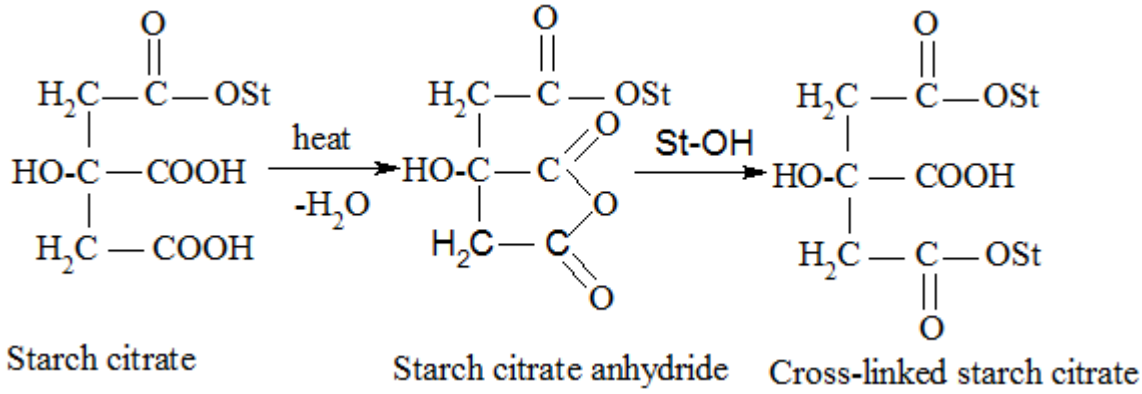

Figure 1.4: Thermochemical reaction of citric acid with starch resulting in cross-linked starch citrate (Fajd and Marton, 2004).

1.3.1.2 Application of Starch Citrate

The thermochemical reaction of starch and citric acid mixture yields starch citrate of high ion binding capacity (Wing and Peoria, 1996; Fajd and Marton, 2004). Starch citrate like traditional ion exchange resins can be used as packing in columns. It responds to the pH of the solution like other weakly acidic cation exchange resins. They are used to remove toxic, heavy metal ions in water purification (industrial waste water) as biodegradable ion-exchange materials against the petrochemically derived ion-exchange resins (Tharanathan, 2005). Its low raw material and production costs together with its outstanding environmental friendly properties can make starch citrate a competitive alternative for traditional ion exchange resins.

Resistant starch (RS) is the starch that is resistant to enzyme digestion and is not absorbed in the small intestine of healthy individuals (Wepner *et al.*, 1999). Digestive enzymes may not be able to completely degrade modified starches because the substituents hinder enzymatic attack and make neighboring bonds resistant to degradation. Hydroxypropyl, acetate, phosphate and citrate starches have been tested for enzymatic degradation. RS displays many physiological benefits of dietary fiber, such as calorie reduction and colonic health benefits including increased stool bulk, decreased transit time of stool, and generation of volatile free fatty acids, especially butyric acid.

Citric acid is rated as nutritionally harmless compared to other substances used for derivatization. The rate of digestion by pancreatin is decreased with increasing degree of

substitution (DS) in starch citrate (Xie and Liu, 2004). RS in the diet is able to lower the glycemic response in humans and, hence, reduce the incidence of type II diabetes (Hoebler *et al*., 1999). Furthermore, RS can be used as a food ingredient to improve cholesterol metabolism and reduce the risk of colon cancer (Yue and Waring, 1998).

Starch from *icacina trichantha* tuber was chemically modified to starch citrate (Omojola *et al*., 2012). Its physicochemical characterization showed that the starch citrate has a better disintegrant activity than its native starch. The starch citrate had a swelling capacity of 16.4 at 85 ^{0}c in water; it did not gelatinize at 100 ^{0}c in water; and had a pH of 4.59. *Tacca involucrate* has also been modified to produce its citrate derivative. It is reported that it has better swelling power and water absorption properties than the native starch. The authors concluded that it is a promising pharmaceutical excipient (Adebiyi *et al*., 2011).

Veerreddy *et al*. (2012) prepared and evaluated potato starch citrate as disintegrant in tablets of wet granulated formulations of diclofenac, cetirizine and paracetamol, employing microcrystalline cellulose (MCC) as diluent and HPMC as binder, and compared with sodium starch glycolate (SSG). The authors concluded that starch citrate exhibited good disintegrating properties as sodium starch glycolate. Chowdary and Enturi (2011a) used starch citrate as a carrier in solid disperssion to improve the dissolution of efavirenz. They further evaluated for pharmacokinetic parameters. The study indicated rapid and higher absorption and bioavailability of efavirenz when administered as solid dispersion in potato starch citrate as a carrier (Chowdary and Enturi, 2013). In another study, Chowdary *et al.* (2011) prepared etoricoxib tablets using potato starch citrate as direct compressible filler to improve dissolution.

1.4 Disintegrating Agents

1.4.1 Overview of Disintegrants

The release of an active ingredient from a tablet or capsule involves two distinct processes: disintegration of the tablet and dissolution of the active ingredient. The two processes are sequential and occur simultaneously until the tablet disintegrates completely (Nelson and Wang, 1977).

Disintegrating agents are substances routinely included in the tablet formulations to aid in the break-up of the compacted mass into the primary particles to facilitate the dissolution or

release of the active ingredients when it is put into a fluid environment. They enhance moisture penetration and dispersion of the tablet matrix (Pahwa and Gupta, 2011). The stronger the binder, the more effective must be the disintegrant for the tablet to release its medication (Mohanachandran *et al*., 2011).

For a medicinal agent in a tablet to become fully available for absorption, the tablet must first disintegrate and discharge the drug to body fluids for dissolution. The general manner a tablet disintegrates is: (A) the tablet wets down, (B) the dissolution liquid penetrates the pore space, (C) the disintegrant absorbs water and swells, and this swelling causes the tablet to break down into granules. Figure 1.5 shows the disintegration pathways of solid dosage forms for the dissolution and absorption of drugs. After the disintegration process, the solid dosage forms change into granules or smaller and fine particles ready for dissolution and absorption.

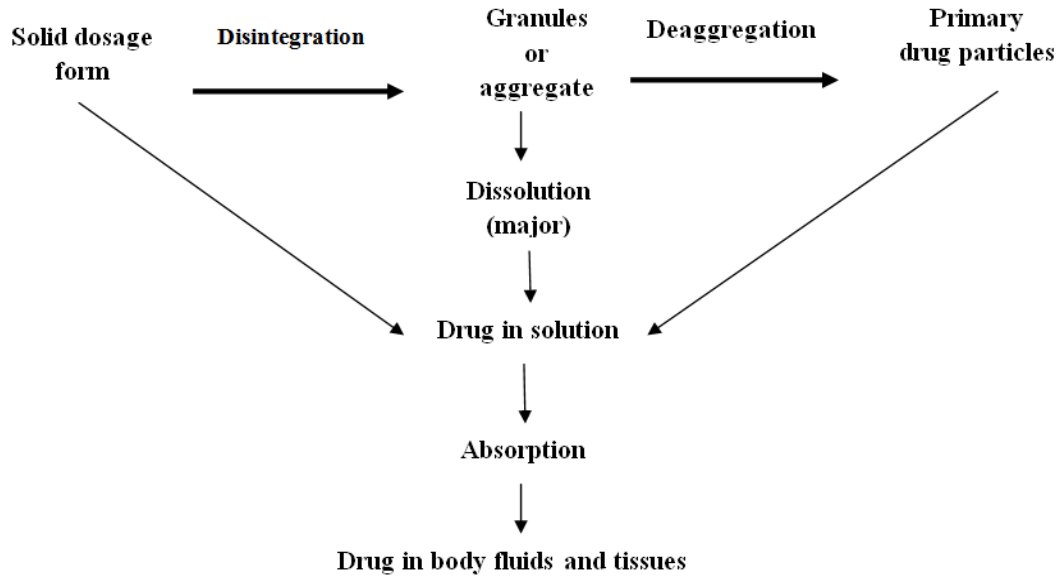

Figure 1.5: Disintegration and dissolution pathways of solid dosage forms (Lee, 2008).

Corn and potato starches were recognized the ingredients in tablet formulations for disintegration as early as 1906. To be effective, corn starch has to be used in concentrations of between 5 - 10%. Below 5%, there are insufficient “channels” available for wicking to take place. Above 10%, the incompressibility of starch makes it difficult to compress tablets of sufficient hardness. Table 1.1 shows the commonly used disintegrants.

Table 1.1: Commonly used disintegrants.

Disintegrant	Normal, (%) concentration	Comments
Starch	5 - 10	Probably works by wicking; swelling minimal at body temperature
Microcrystalline cellulose		Strong wicking action; loses disintegrant action when highly compressed
Insoluble ion exchange resins		Strong wicking tendencies with some swelling action
Sodium starch glycolate[a]	2 - 8	Free-flowing powder that swells rapidly on contact with water
Croscarmellose sodium[a]	1 - 5	Swells on contact with water
Gums- agar, guar, xanthan	< 5	swell on contact with water; form viscous gels that can retard dissolution, thus limiting concentration that can be used
Alginic acid, sodium alginate	4 - 6	Swell like gums but form less viscous gels
Crospovidone[a]	1 - 5	High wicking activity

[a]Often mentioned as superdisintegrants (Lee, 2008).

In evaluating materials for potential use as tablet disintegrants (Augsberger and Hoag, 2008), the test can be divided into those dealing with the physical characterization of the material (presumed to be powders), and those that are likely to be relevant to the disintegrant activity of the material i.e., tests that measure a parameter during or after hydration. The tests used to assess a particular material depend on the predominant mechanism(s) of disintegrant function for that material.

Particle shape and size: Long fibrous particles may be more effective in some circumstances than rounded or irregular particles because fibers may exert their effect over a longer distance through the tablet matrix. For disintegrants where swelling is a predominant mechanism of function, particle size will be important since larger particles swell more than small particles.

Swelling: There are several different aspects to swelling that need to be considered; the rate of swelling, extent of swelling and swelling force. A material that swells quickly generates more force for disruption of the matrix than a slow swelling material. Similarly a material that showed extensive swelling would generate more force than one that swelled only to a limited extent. In actual use, it will be a combination of both rate and extent that generate the swelling force. The extent of swelling can be assessed in two ways: at the level of individual particles

(intrinsic swelling) and swelling of the bulk powder. Intrinsic swelling may be determined using microscope. Bulk swelling may be determined by measuring the change in volume with time of a powder bed in contact with water.

Sedimentation volume: A known weight of a disintegrant is mixed with water in a measuring cylinder and allowed to stand for a specified period. The volume of the hydrated layer of disintegrant is measured. This measures the extent of hydration.

Water penetration into a powder bed: This parameter may be determined using the equipment for determination of bulk swelling. The rate of water penetration is linked to the hydrophilicity of the powder. Using this equipment hydration capacity and rate of hydration can be determined.

1.4.2 Mechanisms of Disintegrant Action

It is believed that no single mechanism is responsible for the action of most disintegrants. But rather, it is more likely the result of inter-relationships between these major mechanisms. The mechanism by which tablets are broken into small pieces and then produce a homogeneous suspension is based on:

A) Swelling: Although not all effective disintegrants swell in contact with water, swelling is believed to be a mechanism in which certain disintegrating agents (such as starch) impart the disintegrating effect. By swelling in contact with water, the adhesiveness of other ingredients in a tablet is overcome causing the tablet to fall apart.

B) Porosity and capillary action (wicking): Effective disintegrants that do not swell are believed to impart their disintegrating action through porosity and capillary action. Tablet porosity provides pathways for the penetration of fluid into tablets. The disintegrant particles themselves act to enhance porosity and provide pathways into the tablet. Liquid is drawn up or "wicked" into these pathways through capillary action and rupture the interparticle bonds causing the tablet to break apart (Gupta and Gaud, 2000; Muazu *et al*., 2012).

C) Heat of wetting: When disintegrants with exothermic properties get wetted, localized stress is generated due to capillary air expansion, which helps in disintegration of tablet. This explanation, however, is limited to only a few types of disintegrants (Singh, 1992).

D) Release of gases: Carbon dioxide is released within tablets on wetting due to interaction between bicarbonate and carbonate with citric acid or tartaric acid. The tablet disintegrates due to generation of pressure within the tablet. These effervescent mixtures are formulated when very rapidly dissolving tablets or fast disintegrating tablet are required.

E) Particle repulsive forces: Non-swelling particle were found to cause disintegration of tablets. The electric repulsive forces between particles are believed to be the mechanism of disintegration and water is required for it. Researchers found that repulsion is secondary to wicking.

F) Deformation recovery: Starch grains are generally thought to be "elastic" in nature meaning that grains that are deformed under pressure will return to their original shape when that pressure is removed. But, with the compression forces involved in tableting, these grains are believed to be deformed more permanently and are said to be "energy rich" with this energy being released upon exposure to water.

G) Enzymatic reaction: Enzymes present in the body also act as disintegrants. These enzymes dearth the binding action of binder and help in disintegration (Pahwa and Gupta, 2011).

1.4.3 Ideal Characteristics of Disintegrants

Preethi *et al*. (2013) reviewed the ideal characteristics of disintegrants as: A) Poor solubility, B) Poor gel formation, C) Good hydration capacity, D) Good molding and flow proporties, and E) No tendency to form complexes with the drugs.

1.5 Solubility and Permeability of Drugs

Nowadays, because of the advance of combinatorial chmistry and high throuput screening, the number of poorly water soluble drug candidates has increased tremendously. More than 40% of newly discovered drugs are poorly water soluble. The solubility in gastrointestinal fluids of a drug is an important factor affecting its bioavailability. Solid drugs administered orally for systemic activity must dissolve in the gastrointestinal fluids prior to their absorption. Drugs are catagorized based on their solubility in milliliters of solvent required for dissolving 1g of solute referring to a temperature between 15 ^{0}c and 25 ^{0}c. If 1 g of solid drug requires more than 10000 ml, this drug is classed as practically insoluble (Ansel *et al.*, 2011). The relative

terms of solubility description for each part of solvent required for one part of solute is given elsewhere (Ansel *et al*., 2011; IP, 2015).

Based up on the key determinats of rate and extent of drug absorption from immediate release solid oral dosage forms such as aqueos solubility, gastro-intestinal permeability and dissolution, the biopharmaceutical classification system (BCS) method of classification classifies drugs into four groups. Solubility class is assigned on the basis of the volume of an aqueous medium required to dissolve the highest dose strength in the pH range of 1-7.5. A drug is classified as highly soluble if the drug can be dissolved in a volume of less than or equal to 250 ml of aqueous media. A drug substance is classified as highly permeable if the extent of absorption in humans is greater or equal to 90% of an orally administered dose based on either mass balance determination or comparision to a reference intravenous dose (Dalton and Yates, 2007). Class I drugs are highly soluble and highly permeable, Class II drugs are poorly soluble but highly permeable, Class III drugs are highly soluble but poorly permeable, and Class IV drugs have poor solubility and permeablity (Figure 1.6).

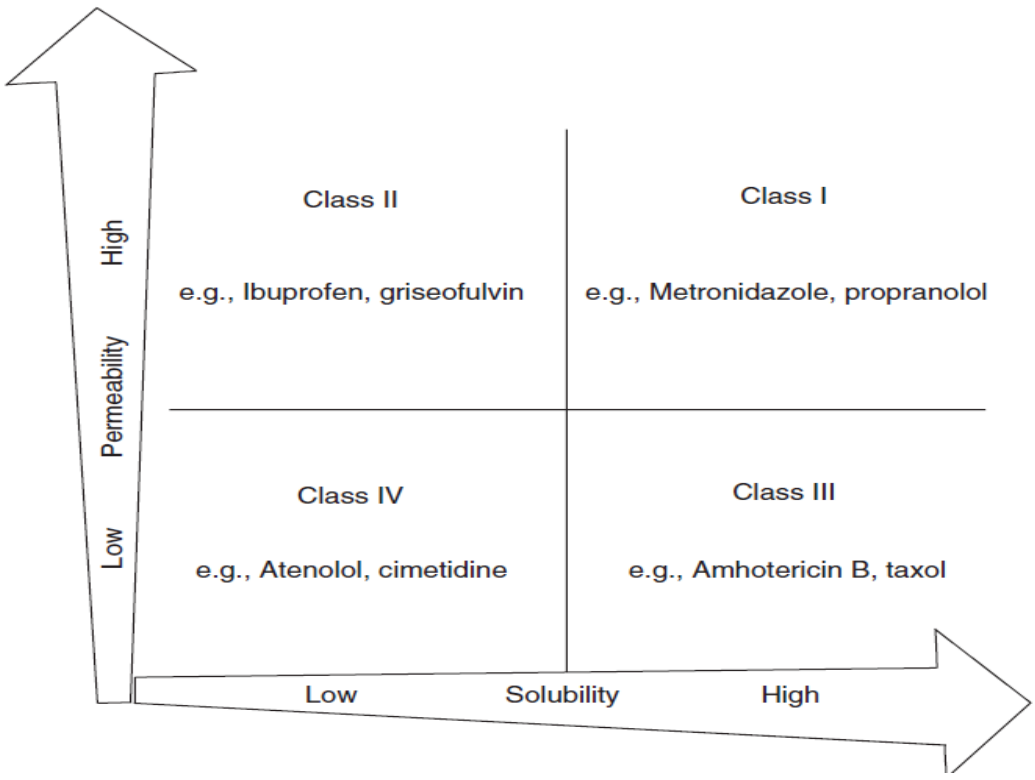

Figure 1.6: Biopharmaceutical classification system (Lee, 2008).

1.5.1 Ibuprofen

Ibuprofen is practically insoluble in water. Based on the biopharmaceutical classification system, it is classified as Class II. It is freely soluble in acetone, in methanol and in methylene chloride (Odeku and Picker-Freyera, 2007; BP, 2009). The chemical structure of ibuprofen is depicted in Figure 1.7. The common analgesic drug, Ibuprofen ($C_{13}H_{18}O_2$), exhibits characteristic dissolution and tableting behavior due to its hydrophobic property (Duerholz *et al., 1992)*. Its high cohesive property results in poor flowability, and during manufacturing it has high tendency of sticking to punches (Bushra *et al.*, 2008).

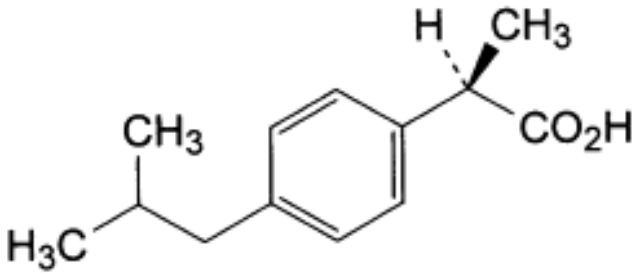

Figure 1.7: Chemical structure of ibuprofen.

In the past, most ibuprofen compressed tablets were prepared by wet granulation method using adhesives, such as starch. Ibuprofen, however, is known to have unique dissolution characteristics when compressed into tablets. One attempt to provide a more stable dissolution profile to ibuprofen compressed tablets is described in U.S. Pat. No. 4,904,477 in which a spray dried of finely divided ibuprofen in a gelatinized starch matrix containing a superdisintegrant, pregelatinized starch, colloidal silica and polyvinylpyrrolidone and/or sodium lauryl sulphate (Duerholz *et al.*, 1992).

1.6 Direct Compression Formulations

Many of the attempts in tablet manufacturing are directed towards the replacement of wet granulation techniques due to the high time and cost burdens they impose on the processes of manufacturing. Consequently emphasis has been given on the use of direct compression due to the simplicity in processing that requires fewer unit operations compared to wet granulation (shorter processing time and lower energy consumption) (Rashid *et al.*, 2013).

The choice of excipient(s) when formulating tablets by direct compression is critical. It must accomplish good compact binding, ability of powder to flow and tablets to disintegrate

rapidly, particle size uniformity, as well as compatibility with other excipients or drugs. Such properties are essential requirements to attain good mixing uniformity and drug delivery by the excipients (Rashid *et al.*, 2013).

Tablet manufacturing by direct compression has increased steadily over the years. It offers advantages over the other manufacturing processes for tablets, such as wet granulation. Direct compression is more economic, reduces the cycle time and is straight forward in terms of good manufacturing practice requirements. On the other hand, wet granulation not only increases the cycle time, but also has certain limits imposed by thermolability and moisture sensitivity of the active. The unnecessary exposure of any drug to moisture and heat can never be justified. Tablets produced by direct compression method give lower microbial levels than those prepared by the wet granulation method. The tablets prepared by direct compression disintegrate into API particles instead of granules that directly come into contact with the dissolution fluid and exhibit a comparatively faster dissolution (Bushra *et al.*, 2008). The use of direct compression as a manufacturing technique is estimated to be as high as 50% of formulations in the United States (McCormick, 2005)

1.7 Optimization methods

Traditionally, experimentation on tablet formulation development are performed by changing the levels of each factor at a time, in an unsystematic way, keeping all other variables constant in order to study the effects of that specific variable on the selected response or to find the optimal conditions of a complete system. This methodology (trial and error) is based on a large number of experiments and often relies merely on the analyst's experience (Kincl *et al.*, 2005).

It is in the best interest of the pharmaceutical scientist to understand the theoretical formulation and target processing parameters, as well as the ranges for each excipient and processing parameter. It is the responsibility of the development team to identify, and set control limits for, the attributes that are found to be critical to the quality of the product (Van Buskirk *et al.*, 2014). Optimization techniques provide both a depth of understanding and an ability to explore and defend ranges for formulation and processing factors. With a rational approach to the selection of the several excipients and manufacturing steps for a given product, one qualitatively selects a formulation. It is at this point that optimization can

become a useful tool to quantitate a formulation that has been qualitatively determined (Schwartz *et al.*, 2002).

Conventional methods of studying a process by maintaining other factors involved at unspecified constant levels does not depict the combined effect of all the factors. Design of experiments (DoEs) provides statistical models, which help in understanding the interactions among the parameters that have been optimized. Response surface methodology (RSM) is one of the experimental designing methods which can surmount the limitations of conventional methods collectively (Gooding, 2004; Ramakrishna and Susmita, 2012). RSM is a collection of mathematical and statistical techniques for empirical model building. By applying a multivariate design for the screening experiments, many excipients are evaluated using comparatively few experiments. The application of RSM to design optimization is aimed at reducing the cost of expensive analysis methods and their associated numerical noise.

Most applications of RSM are sequential in nature. In the DoE approach, first process variables are "screened" to determine which are important to the outcome (excipient type, percentage, mixture time, etc.). The next step is optimization, when the best settings for the important variables are determined. After this evaluation of the variables that have the largest influence on the result are selected for new studies. Thus, a large number of experimental variables can be investigated without having to increase the number of experiments to the extreme (Lundstedt *et al.*, 1998; Zhen *et al.*, 2013).

Thus, RSM and DoE enable to explore the behavior of a formulation by changing the concentration of the excipients, drug substance and process parameters according to today's requirements of ICH Q8 (FDA, 2004). To approximate a response function to experimental data that cannot be described by linear functions, experimental designs for quadratic response surfaces should be used, such as three-level factorial, Box–Behnken, central composite, and Doehlert designs *(*Bezerra *et al.*, 2008).

1.8 The Present Study

Several studies done over the last decades indicate that enset starch has a promising industrial potential. Sodium starch glycolate (SSG) from enset starch was prepared and found to be superior to that of Primojel® (Gebre-Mariam *et al.*, 1996). The SSG from enset starch was investigated as an effective gelling agent for topical gel preparations (Gebriel *et al*., 2013).

The drug sustaining ability of phosphate cross-linked enset starch microspheres has also been investigated (Belachew and Gebre-Mariam, 2009).

Several studies indicate that citric acid modification of starch enhances swelling and hydration capacity of starch (Adebiyi *et al*., 2011; Chowdary and Enturi, 2011a; Chowdary and Enturi, 2011b; Omojola *et al*., 2012; Vikram *et al.*, 2012). These improved properties suggest the potential application of starch citrate as a disintegrant. So far, no work has been done on enset starch citrate as a pharmaceutical disintegrant. Thus, the aim of the present study is to prepare and evaluate enset starch citrate as a disintegrant in tablets employing ibuprofen, a Class II drug according to biopharmaceutical classification system, by direct compression method of tableting using microcrystalline cellulose (MCC) as filler-binder and compare to sodium starch glycolate.

1.9 Objectives

1.9.1 General Objective:

To prepare enset starch citrate and investigate its disintegration capacity in tablet formulations.

1.9.2 Specific Objectives:

- To prepare enset starch citrates (ESCs) and potato starch citrates;
- To characterize the starch citrates (SCs);
- To evaluate the SCs as disintegrants in tablets prepared by direct compression method;
- To compare the disintegration efficiency of ESC with sodium starch glycolate in ibuprofen tablets;

2 EXPERIMENTAL

2.1 Materials

Bulla was purchased from local market in Welliso town, Oromia region, Ethiopia. Citric acid (UNI-CHEM® Chemical reagents, India), benzene (Analyticals® CARLO ERBA: Milano, Italy), potassium dihydrogen phosphate (Sörensen, Leuren, Denmark), sodium hydroxide (Loba Chemie Pvt. Ltd., Mumbai, India), Sodium metabisulfite, hydrochloric acid, MCC and stearic acid (BDH Chemicals Ltd, Poole, England), ibuprofen (China Associate Co Ltd, China), potato starch BP (BDH LTD., Poole, UK), and magnesium stearate (Bulvinos Chemicals Ltd, England) were used as received. All other chemicals were of analytical grade.

2.2 Methods

2.2.1 Enset Starch Isolation

Starch isolation was carried out following the method described by Gebre-Mariam and Schmidt (1996b). Briefly, bulla was suspended in large quantities of distilled water (DW) containing 0.075% (w/v) of sodium metabisulphite. The material was allowed to settle and the supernatant decanted. The sedimented starch was repeatedly treated with sodium metabisulphite solution until the suspension became translucent. The material was then passed through fine muslin to remove cell debris and the translucent suspension was collected and allowed to settle. The sedimented starch was washed several times with DW followed by sieving after each washing until the wash water was clear and free of suspended impurities. The resulting starch was dried in air at room temperature and milled gently to fine powder with mortar and pestle after which it was sieved and made ready for further processing.

2.2.2 Preparation of Starch Citrate

Enset starch citrate, ESC, was prepared following the method described by Chowdary *et al* (2011) with slight modifications. Citric acid, CA (10 g) was dissolved in 10 ml of DW, the pH of the solution was adjusted with 10M sodium hydroxide solution to 3 or 3.5 pH and finally the solution made up to 25 ml with DW. The CA solution (25 ml) was mixed with 25 g of native enset starch (NES) in a beaker and the contents were poured on to a tray. The tray was then placed in open air for conditioning for 18 h or dried at 50 ^{0}C in an oven for 24 h, and both further dried in an oven for 6 h at 60 ^{0}C. To facilitate the reaction, the mixture obtained

was ground and baked at 120 ^{0}C or 130 ^{0}C for 2 h in an oven. The baked mixture was then repeatedly washed with distilled water to remove unreacted citric acid. The washed starch citrate, hereinafter designated as SC, was dried in an oven at 50 ^{0}C for 72 h to remove the moisture after which the product was ground. Potato SC was also prepared for comparison. The design of the reaction conditions is given in Table 2.1.

Table 2.1: Reaction conditions for the modification of enset and potato starches for a duration of 2 h.

Type of starch	pH of medium	Reaction temp (^{0}C)	Drying condition	Designation
Potato	3.0	120	M'	P3120M'
Potato	3.0	130	M'	P3130M'
Potato	3.5	120	M'	P3.5120M'
Potato	3.5	130	M'	P3.5130M'
Enset	3.0	120	M'	E3120M'
Enset	3.0	130	M'	E3130M'
Enset	3.5	120	M'	E3.5120M'
Enset	3.5	130	M'	E3.5130M'
Potato	3.0	120	M	P3120M
Potato	3.0	130	M	P3130M
Potato	3.5	120	M	P3.5120M
Potato	3.5	130	M	P3.5130M
Enset	3.0	120	M	E3120M
Enset	3.0	130	M	E3130M
Enset	3.5	120	M	E3.5120M
Enset	3.5	130	M	E3.5130M

M' represents conditioning at room temperature (21±2 ^{0}C) for 18 h and then drying in an oven at 60 ^{0}C for 6 h; and M represents drying at 50 ^{0}C for 24 h and then at 60 ^{0}C for 6 h.

2.2.3 Characterization of the SCs

2.2.3.1 Moisture Content

Moisture content was determined following the method described in USP-30/NF-25 (2007) for modified starches. Accordingly, 2 g of each of the samples was weighed into previously washed, dried and weighed petri-dish and was heated in an oven (Kottermann® 2711, H. JURGENS & CO., Bremen, Germany) at a temperature of 120 ^{0}C for 4 h. The sample was then weighed, and the moisture content expressed from triplicate determinations using Eq. 2.1.

$$\%\ \text{Moisture content} = \frac{Wi - Wf}{Wi} * 100 \qquad (2.1)$$

where W_i and Wf are the weights of NES/SCs before and after drying, respectively.

2.2.3.2 Degree of Substitution (DS) and Esterification (ED)

The DS and ED were determined following the method described by Shi *et al*. (2007). The NaOH and HCl volumetric solutions were prepared according to Japanese Pharmacopeia15th edition (JP, 2006). One gram (1 g) from each of the samples of SCs was dissolved in dimethylsulphoxide (DMSO) in a conical flask and then excess of standardized 0.186N NaOH was added to the solution to saponify the ester. The excess unreacted NaOH was determined by titration with the standardized 0.226N HCl to phenolphthalein end point. Eq. 2.2 was used to calculate the ED as:

$$\mathrm{ED} = (\mathrm{VNaOH} * \mathrm{NNaOH} - \mathrm{VHCL} * \mathrm{NHCL})\mathrm{G} * \frac{100}{\mathrm{m}} \qquad (2.2)$$

Where VNaOH and VHCl are the volumes (in L) of the NaOH solution and HCl solution, and NNaOH and NHCl are their normal concentrations (mol/l). G is the weight addition of one glucose ring unit which was substituted by the CA, and the value of G is 174 (g/mol). 'm' is the weight of the sample in grams. The DS was calculated according to Eq.2.3.

$$\mathrm{DS} = 162 * \frac{\mathrm{A}}{1 - \mathrm{G} * \mathrm{A}} \qquad (2.3)$$

where A is the mole number of the NaOH solution which reacted with 1 g substitute; 162 is the molecular weight of the glucose ring unit. The results for ED & DS are the mean of three determinations.

2.2.3.3 Settling Volume

The determination of settling volume was based on the method used for croscarmellose sodium in the USP 30/NF 25 as used by Bele and Derle (2012) with slight modification. Swelling medium (20 ml) was added to a 25 ml graduated cylinder. The sample (0.5 g) was added to the medium, shaking vigorously after addition. The volume was brought to 25 ml and shaken until all the powder was homogenously distributed. The volume of the settled mass was measured at the end of 4 h. To determine the effect of medium pH on settling volume, the study was performed in distilled water (DW), 0.1N HCl and simulated salivary fluid (SSF) (pH 6.8) at room temperature. Samples were analyzed in triplicates.

To study the effect of magnesium stearate on the settling volume of the SCs, E3.5130M' was selected and different concentrations of magnesium stearate were used in the study (Bele and Derle, 2012). Three grams of the SC was mixed with different levels of magnesium stearate in 20 ml glass container using Turbula mixer (Willy A. Bachofen AG, Turbula 2TF, Basel, Switzerland) at 49 rpm for 5 min. Settling volume was determined from 1 g sample in DW.

2.2.3.4 Gelling Properties

The gelling properties of the NES and ESCs were evaluated following the method described by Chowdary *et al.* (2011). The gelling property of the starch and ESC prepared was evaluated by heating 7% dispersion in water at 100 ^{0}C for 30 min after which the flow property of the sample after 24 h was observed by inverting its container at 45^{0}.

2.2.3.5 Hydration Capacity

The Hydration capacity (water retention capacity) was determined following the method used by Iwuagwu and Onyekweli (2002). One gram of powder was put in each of three centrifuge test tubes and suspended in 10 ml of DW. The tube was shaken intermittently every five min over a 2 h period and left to stand for 30 min. It was then centrifuged for 15 min at 3000 rpm and the supernatant was decanted and the hydration capacity determined by Eq. 2.4 as:

$$\text{Hydration Capacity} = \frac{x}{y} \tag{2.4}$$

where x is weight of hydrated powder after centrifugation and decanting, and y is weight of dry powder. Results are expressed as a mean of three determinations.

2.2.3.6 Swelling Power and Solubility

Swelling power (SP) and water solubility index (WSI) of the samples were determined following the method described by Odeku and Picker-Freyera (2007) with slight modifications. Sample suspensions were prepared by dispersing 0.30 g of NES/SC samples in 10 ml DW in pre-dried and weighed centrifuge test tubes and heated to room temperature, 37 ^{O}C, 55 ^{O}C, 65 ^{O}C, and 85 ^{O}C for 30 min with shaking every 5 min and then left to cool to room temperature. The suspensions were centrifuged for 15 min at 3000 x g. The supernatant was decanted and dried in an oven for 2 h at 130 ^{O}C. The residue obtained after drying the supernatant (W1) represents the amount of starch solubilized in water at that particular temperature. The residue obtained was weighed (Ws) to obtain the swelling of the starch. The

WSI and SP were determined according to Eqs. 2.5 and 2.6, respectively. Results are expressed as a mean of three determinations.

$$\mathrm{WSI}(\%) = \frac{\mathrm{W1x100}}{0.5} \tag{2.5}$$

$$\mathrm{SP} = \frac{\mathrm{Wsx100}}{0.5(100 - \mathrm{WSI})} \tag{2.6}$$

2.2.3.7 Moisture Sorption Capacity

Moisture sorption capacity of the NES/SC was determined following the method described by Forney and Brandl (1992) with slight modification. Pyrex desiccators containing distilled water, or appropriate solutions of glycerol were prepared to provide different RH chambers at room temperature. The samples were pre-dried in an oven (Kottermann® 2711, H. JURGENS & CO. GmbH & CO., Bremen, Germany) for 4 h at 120 ^{0}C. Two grams (W) of the samples were spread evenly on each dried and weighed petri-dish and transferred to particular relative humidity chambers. Samples were equilibrated for four (4) weeks at room temperature. The weights after 4 weeks were recorded and the moisture content of each sample was calculated as the weight difference of the samples before and after equilibration (W_g) in a given relative humidity. Moisture sorption capacities (Wa) of the NES/SCs are expressed as a mean of three measurements of percent moisture sorbed as calculated from Eq. 2.7.

$$\mathrm{Wa} = \frac{(\mathrm{Wg} - W)}{W} * 100 \tag{2.7}$$

2.2.3.8 Morphological Characterization

The starch/SC granule morphology was studied following the method described by Gebre-Mariam and Schmidt (1996b). The sample was mounted and sputter coated with gold to a thickness of about 30 nm (Sputter Coater Type E 5100, Biorad GmbH; Munich, Germany) to increase their conductance. Scanning electron micrographs were taken with a DSM 940 SEM (Carl Zeiss, Oberkochen, Germany) at an accelerating voltage of 5 KV. The samples were then viewed and photographed.

2.2.3.9 Determination of Flow Properties

A) Determination of Density and Related Properties

Bulk density

Bulk densities of the NES and SCs samples were determined by carefully pouring 30 g powder into a 250 ml graduated glass measuring cylinder. The cylinder was then lightly tapped twice to collect all the powder sticking on the wall of the cylinder. The volume was then read directly from the cylinder and used to calculate the bulk density. The bulk density (g/cc) was calculated by using Eq. 2.8.

$$Bulk\ density(\rho b) = \frac{m}{Vb} \tag{2.8}$$

Where *m* is the weight of the powder and *Vb* is bulk volume.

Tapped density

For the determination of tapped density, 30 g of powder was tapped in graduated measuring cylinder 500 times using tapping densitometer (ERWEKA Gmbh, Heusenstamm, Germany) to attain a constant volume reading from the cylinder and the tapped density was calculated from the weight and tapped volume of the powder by using Eq. 2.9. Tapped density (g/cc) was determined as a mean of three measurements.

$$Tapped\ density(\rho b) = \frac{m}{Vt} \tag{2.9}$$

Where m is weight of powder and Vt is volume of the tapped powder.

Carr's index (CI) and Hausner's ratio (HR)

The Carr's index was calculated from the difference between the tapped densities and the bulk densities divided by the tapped densities and multiplied by hundred. The Hausner's ratio is the ratio of the tapped and bulk densities. Carr's index and Hausner's ratio were calculated according to Eqs. 2.10 and 2.11 respectively.

$$Carr's\ index = \frac{tapped\ density - bulk\ density}{tapped\ density} \times 100 \tag{2.10}$$

$$Hausner's\ ratio = \frac{tapped\ density}{bulk\ density} \tag{2.11}$$

True Density

The true density (Dt) of the NES/ESC was determined by liquid displacement method using benzene as displacement liquid, following the method described elsewhere (González and Pérez, 2002). NES/SC sample (2 g) was placed in a pycnometer, closed and weighed (the weights of the empty pycnometer and of the pycnometer filled with benzene were measured). Sufficient benzene was added to wash down and overlay the sample. To release entrapped air the content of the pycnometer was gently stirred with glass rod. The sample was allowed to settle, filling the pycnometer with benzene. After equilibrating the pycnometer for 15 min at room temperature, the meniscus was adjusted and weighed. The Dt was determined as a mean of three measurements using Eq. 2.12.

$$\mathrm{Dt} = \frac{WS}{a+w-b} \tag{2.12}$$

where W: weight of NES/ESC, S: specific gravity of benzene, w: weight of pycnometer filled with benzene and b: weight of pycnometer plus benzene plus NES/SC.

B) Angle of Repose

The angle of reposes was determined by fixed funnel method. A glass funnel having a 10 mm aperture was mounted on a laboratory stand at appropriate height to adjust the tip of the funnel becomes 3 cm above the tip of the heap of powder (JP, 2006) and 30 g of the sample was poured into the funnel gently from cylinder. The height, h and diameter, 2r (radius), of the sample heap was measured. The angle of repose was calculated according to Eq. 2.13.

$$Angle\ of\ repose\ \theta = tan^{-1}(\frac{h}{r}) \tag{2.13}$$

2.2.4 DSC Study of Drug-excipient Compatibility

DSC 200 thermal analyzer (Netzsch-Geralebau Gmbh, Selb, Germany) was used to study the compatibility of ibuprofen with the SC. DSC analysis of the SC (E3.5130M' was taken, because it has relatively better disintegrating effect), ibuprofen and their 1:1 mixture were performed. The samples were weighed and encapsulated in a flat bottomed aluminum pans. Liquid nitrogen was used as coolant. The samples were scanned at 10 ^{0}C per min over temperature range of 0 ^{0}C to 250 ^{0}C.

2.2.5 Tablet Formulation and Tableting

2.2.5.1 Preparation of Powder Mixture for Direct Compression.

Ibuprofen, MCC, colloidal silicon dioxide, talc, ESC and magnesium stearate were passed through a sieve of pore size 315 μm. Weighed amounts of ibuprofen, MCC, colloidal silicon dioxide or talc and ESC (Table 2.2) were mixed for 15 min in Turbula mixer (Willy A. Bachofen AG, Turbula 2TF, Basel, Switzerland) at 49 rpm and their powder characteristics were studied. Results were reported as a mean of three measurements for preliminary selection of best combination based on flowability, compressibility and tablet characteristics.

Table 2.2: Blend composition for screening of better glidant and tablet weight for the direct compression formulation of ibuprofen tablets.

	A		B		C		D	
Item	%	Amount mg/tab	%	Amount mg/tab	%	Amount mg/tab	%	Amount mg/tab
Ibuprofen 200mg	52.63	200.00	50	200.00	52.63	200.00	50	200.00
Avicel PH 102	43.87	166.7	46.5	186	42.37	161	45	180
ESC	2	7.6	2	8	2	7.6	2	8
CSD	0.5	1.9	0.5	2				
Talc					2	7.6	2	8
MgS	1	3.8	1	4	1	3.8	1	4
Total	100	380	100	400	100	380	100	400

A, B, C and D are formulations; CSD: Colloidal silicon dioxide; MgS: magnesium stearate

2.2.5.2 Determination of Density and Related Properties, and Angle of Repose

The blends were characterized for bulk and tapped densities, Carr's index and Hausner's ratio, and angle of repose, and results were calculated in the same way as described for SC powders above (Sections 2.2.3.9 A and B) by Eqs 2.8, 2.9, 2.10, 2.11 and 2.13, respectively.

2.2.5.3 Compression of the Mixture intoTablets

The mixture, after mixing with 1% magnesium stearate for five min, was compressed into 380 mg or 400 mg total tablet weight (Wt) which contains 200 mg ibuprofen with compression

forces (CF) yielding tablets of hardness between 50 N and 120 N. Screening was conducted on two glidant types and two total tablet weights (Table 2.2).

After screening for weight of tablet and type of glidant, series of trials were conducted to attain working CFs and disintegrant concentrations (DisCs) for the optimization process of the formulation. Compression of the selected composition was conducted by the use of Korsch XP1 single punch (Korsch XP1; Berlin, Germany) tablet press with 10 mm punch size at 3 levels of CF and (DisC), respectively at 4 KN, 7 KN & 10 KN and 2%, 3% & 4%, after mixing with 1% magnesium stearate for 5 min in Turbula mixer. The produced tablets were stored in airtight bottles for 24 h at room temperature before further analysis for physical characteristics.

2.2.6 Evaluation of Tablet Physical Characteristics

2.2.6.1 Weight and Thickness

Twenty (20) tablets were randomly selected from each batch and weighed individually on an analytical balance (ADAM, Adam equipment, Milton Keynes, UK). Tablet thickness was measured using sliding caliper scale (Nippon, Sokutei, Japan).

2.2.6.2 Hardness

After 24 h of production, ten tablets were taken from each batch and/or run at random and the hardness of the tablets was determined using hardness tester (Schleuniger, 2E/205, Switzerland).

2.2.6.3 Friability

Twenty (20) tablets were weighed from each batch and/or run and then placed in a friability tester (ERWEKA, Gmbh, Heusenstamm, Germany). The friability tester was subjected to revolve at 25 rpm for 4 min. The tablets were then dedusted and weighed, and the percent loss in weight was calculated as friability.

2.2.6.4 Disintegration time

Disintegration test was carried out according to USP 30/NF 25 (2007) specifications. Tablets from each batch were placed in a disintegration tester (CALEVA, G.B. Caleva Ltd., UK).

2.2.7 Standard Calibration Curve of Ibuprofen

Stock solution containing 100 µg/ml of ibuprofen in phosphate buffered solution (pH 7.2) was prepared. Different concentrations (1, 2, 4, 6, 8 and 10 µg/ml) were prepared from the stock solution. The UV absorbance readings were taken at 221 nm using spectrofluorimeter CM2203 (SOLAR; Belarus, Minsk). The absorbance of the solution as a function of its concentration was plotted and a calibration curve with linear regression equation constructed.

2.2.8 Dissolution Profile

The dissolution test was done according to the USP 30/NF 25 (2007) specification using dissolution apparatus Type II (ERWEKA, DT600, Germany), with 900 ml phosphate buffer (pH 7.2) as the dissolution medium at 37 ± 0.5 ^{0}C and at a stirring rate of 50 rpm. Five ml of aliquots of the dissolution medium were removed at 5, 10, 15, 20, 30, 45 and 60 min and filtered using Whatman No 4 filter paper. Equal amount of fresh medium kept at the same temperature was added into the dissolution vessel. One ml of the filtered samples was diluted to 100 ml and absorbance of the samples was measured at 221 nm in a spectrofluorimeter CM 2203 (SOLAR, Close Corporation; Minsk, Belarus).

2.2.9 Statistical Design and Analysis

A full 3-level factorial design was used in optimizing the direct compression formulation of ibuprofen tablets to estimate the influence of individual variables (main effects) and their interactions. To determine the range of DisC and CF, series of preliminary experiments were carried out in compressing ibuprofen tablets. DT, hardness and friability were taken as responses in the experiment. Ibuprofen was made constant at 200 mg per tablet. Magnesium stearate was employed at 1% level. Weight of tablet and type of glidant were determined from the preliminary studies carried out. MCC was used as filler-binder in the formulation.

All the data measured and reported in this study are averages of three or more measurements and the values are expressed as mean ± standard deviation (SD). Values in parentheses are SDs. Wherever appropriate, the data was subjected to further statistical analysis making use of software packages, one way analysis of variance (ANOVA), etc. Statistical analysis was performed by using Microsoft Excel and Origin 7 software (Origin Lab Corporation, MA, and USA), and plots of swelling power, solubility, settling volume, moisture sorption and calibration curve are constructed using Origin 7 software. To demonstrate the influence of

each factor on responses and to indicate the optimum level of factors, contour and 3D surface plots were generated using Design-Expert® software (V 9.0.6.2; Stat-Ease Inc., Minneapolis, MN, USA). Significance test, $p < 0.05$ was considered significant.

Three-level factorial design in the RSM was used to design the experiments with two replicates (Singh *et al*., 2013). The total number of runs is 11: 4 factorials, 3 centers and 4 centedge. The complete design of the experiments is given in Table 2.3.

Table 2.3: Three level factorial matrix in terms of coded and actual factors levels for factors CF and DisC for the directly compressed formulation of ibuprofen tablets.

Run	Coded level		Actual level	
	CF (KN)	DisC (%)	CF (KN)	DisC (%)
F_1	1	-1	10	2
F_2	0	0	7	3
F_3	-1	0	4	3
F_4	0	0	7	3
F_5	-1	1	4	4
F_6	0	0	7	3
F_7	0	1	7	4
F_8	1	1	10	4
F_9	-1	-1	4	2
F_{10}	1	0	10	3
F_{11}	0	-1	7	2

All model formulations were treated by Design-Expert® software in analyzing the responses. The best fitting mathematical model was selected based on the comparisons of several statistical parameters including the coefficient of variation (CV), the multiple correlation coefficient (R^2), adjusted R^2, the predicted residual sum of square (PRESS), and lack of fit (LOF) proved by Design-Expert® software (Huang *et al.,* 2005).

3 RESULTS AND DISCUSION

3.1 Preparation of Enset Starch Citrate

Under the reaction conditions given in Table 2.1, the substitution agent for the reaction is essentially citric acid. Initially, water and NaOH probably cause swelling of the starch and make the functional groups exposed for reaction with the citric acid. The starch becomes very viscous and cohesive. To decrease the moisture content, the starch was dried at room temperature or at 50 ^{0}C to examine moisture effect on modification. To effect the reaction, the mixture was subjected to high temperature at 120 ^{0}C or 130 ^{0}C. According to reports, lower reaction tempratures result in incomplete reaction and higher temperatures in cross-linking of the starches. To remove unreacted residue of the citric acid, the SCs were washed several times. To significantly reduce the moisture level, the SC was then dried for 72 h, partly because it is hygroscopic (and since it will be used in a moisture sensitive ibuprofen tablet preparation).

When citric acid is heated, it will dehydrate to yield the anhydride form. The citric anhydride can then react with starch to form starch citrate (Figure 1.2). A concentration of 40% citric acid based on starch dry weight was reported to be optimum in the citric acid-starch reaction mixture (Xie and Liu, 2004). In this study also, 40% citric acid based on starch dry weight was used to modify enset and potato starches to produce the SCs.

3.2 Characterization of the SCs

Considering the physical properties of tablet excipients may be crucial for bioavailability and dosage form stability and brand-to-brand variations, and lot-to-lot reproducibility of such properties deserve careful considerations. As for tablet disintegrants, the appearance on the pharmaceutical market of the new so-called superdisintegrants, whose efficiency is strictly related to the preparative techniques, has demonistrated the need for specifications reflecting their performance. Properties closely related to the disintegration efficiency are water uptake, particle swelling and particle dimensions (Caramella *et al*., 1980).

3.2.1 Moisture Content

Excipients can affect drug stability by being a source of moisture. For example, owing to the high moisture content of polyvinylpyrrolidone and urea, aspirin hydrolysis was enhanced in

solid dispersions with these excipients. Decreased drug stability caused by excipients having higher moisture-containing ability has been reported for tablets of aspirin and ascorbic acids, a urea-linoleic acid inclusion complex, powders of cysteine derivatives, and dry syrups of cephalexin (Yoshioka and Stella, 2002).

Moisture is known to affect a wide range of physico-mechanical properties of pharmaceutical formulations including powder flow, compressibility/compactibility, hardness of granules and tablets, die-wall friction and stability (physical, chemical and microbiological) (Hoag *et al.*, 2008). Reduction in moisture content reduces chances of microbial spoilage and hydrolysis thereby increasing the stability and shelf-life of the derivatives (Kemas *et al.*, 2012). Regulation of moisture in formulation is very important as high moisture content may interfere with active ingredient (Hoag *et al.*, 2008). Because ibuprofen tablets are very susceptible to moisture contents, the SCs have been dried for 72 h at 50 ^{0}C in an oven and their moisture contents were reduced to a great extent. Result of moisture content for NES and the SCs is given in Table 3.1. NES has higher moisture content than the SCs because NES is dried in air in contrast to the oven dried SCs.

3.2.2 Degree of Substitution and Esterification

The degree of substitution (DS) is a common method of characterizing starch derivatives and is a measure of the average number of hydroxyl groups on each D-glucopyranosyl unit. It is expressed as the moles of substituent per D-glucopyranosyl units, and the maximum DS is 3 since three hydroxyl groups are available in the unit for substitution. Most commercially produced derivatives have a DS less than 0.2 (Newman *et al.*, 2007).

The chemical and functional properties achieved when modifying starch by chemical substitution depend on the starch source, reaction conditions (reactant concentration, reaction time, pH and the presence of catalyst), type of substituent, extent of substitution (DS), and the distribution of the substituent in the starch molecule (Odeku and Picker-Freyer, 2009).

The result of DS and ED of the SCs obtained from modification of NES with citric acid is shown in Table 3.1. The DS of E3120M' (0.19) is significantly higher ($p < 0.05$) than that of E3.5130M' (0.11) and P3.5130M' (0.10). Xie and Liu (2004) reported an increase in DS from 0.09 to 0.12 when reaction time increased from 3 h to 7 h, then dropped to 0.09 when the

reaction time reached 9 h at a modification temperature of 140 ^{0}C for maize starch indicating dissociation of the substituent from starch when the reaction time extends beyond 7 h.

3.2.3 Gelling Properties

The gelling property of the starch and ESC prepared was evaluated by heating 7% dispersion in water at 100 ^{0}C for 30 min after which the flow property of the sample after 24 h was observed. The ESC flowed easily while the native starch gelled and had no tendency to flow. Poor gel formation is an ideal characteristic of a disintegrant (Preethi *et al.,* 2013). The SCs do not gelatinize at even higher temperature; this indicates that its absorption of water at all tempratures is reversible. This is in-line with other observations conducted (Adebiyi *et al.*, 2011; Chowdary and Enturi, 2011a; Chowdary and Enturi, 2011b; Omojola *et al.*, 2012; Vikram *et al.,* 2012)

3.2.4 Hydration Capacity

From Table 3.1, it can be seen that the water absorption capacity of starch citrate is significantly higher than that of the native starch. This may be a desirable property for the modified starch in the pharmaceutical industry (Omojola *et al*., 2012).

Patel and Vavia (2010) developed cross-linked polyvinyl alcohol with excellent disintegration properties comparable to Ac-Di-Sol. This disintegrant was found to be effective at a concentration ranging from 2-5% w/w. The lower value for sedimentation volume and hydration capacity suggested its limited swelling potential as compared to Ac-Di-Sol, SSG and crospovidone. In another study, the disintegrating capacity of starch phosphates, and starch-urea-citrate complex had been evaluated to have higher hydration capacity compared to native starch. The authors suggested that the high hydration capacity was the proposed reason for the disintegration ability.

Substitution of cassava starch with citrate groups brought about significant increase in the water binding capacity (Jyothi *et al.*, 2007). The increase in water binding capacity of the SCs can be attributed to the breakage of H-bonds and inclusion of substituent groups in the starch structure, which allows easy penetration of water molecules.

The results of hydration capacities of the NES, SCs and SSG are given in Table 3.1. The hydration capacity of the SCs indicates that the SCs are capable of absorbing more water than

the NES. The study of swelling capacity, which reflects increase in volume of the SCs after in contact with water, showed E3.5120M has the highest increase in volume among the ESCs (Hasan *et al*., 2012). Among the examined samples, the decreasing order of hydration capacity is: SSG > E3.5120M > E3.5130M' > E3120M' > NES.

Table 3.1: Moisture content, ED, DS and hydration capacity of NES and SCs.

Item	Moisture content (% g/g)	ED	DS	Hydration capacity (g/g)
NES	14.2±1.7	NA	NA	1.86±0.02
E3.5130M'	5.04±0.26	10.14±0.11	0.11±0.00	6.43±0.03
E3120M'	5.71±0.01	16.59±0.11	0.19±0.00	5.64±0.03
P3.5130M'	5.11±0.54	9.81±0.20	0.10±0.00	
E3.5120M				7.72±0.12
SSG				11.71±0.20

SSG: Sodium starch glycolate; NA: not applicable.

3.2.5 Settling Volume

Zha and Augsburger (2005) demonstrated that acidic medium significantly reduces the liquid uptake rate and capacity of sodium starch glycolate (Primojel) and croscarmellose sodium (Ac-Di-Sol) but not crospovidone NF (Polyplasdone XL10). Caramella *et al*. (1980) determined that the differences in swelling ability among various disintegrants are far less marked in acidic medium than in isotonic saline. An increase in the pH of the medium from acidic to neutral increased the bulk swelling of the particles of polacrilin potassium (Bele and Derle, 2012).

The swelling of some disintegrants is dependent on the pH of the medium they are in. A significant reduction in swelling capacity is observed for Ac-Di-Sol in 0.1 N HCl, which swells to half that in water. The strong decrease in swelling capacity of chemically modified starches and celluloses may attribute to the converting of the carboxymethyl sodium moieties to its free acid form in acidic medium for both substances. Since the acid form has less hydration capacity than its salt form, the liquid holding capacity of the disintegrant particles reduces after deionization in the acidic medium.

Shangraw *et al.* (1980) reported that sedimentation volumes of anionic cross-linked starches and celluloses are altered in acidic media. Polyplasdone XL® and Starch 1500® were

unchanged. In a separate study, Chen *et al.* (1997) showed that acetaminophen tablets containing Primojel and Ac-Di-Sol have longer disintegration and dissolution times in acidic than in neutral medium. Those containing Polyplasdone XL showed no such differences.

In this study, the settling volume of the samples was higher in DW followed by SSF and 0.1N HCl (Figure 3.1). The results obtained are in agreement with those obtained by Bele and Derle (2012). This is in line with the fact that they remain unionized in acidic pH, given that the SC is a salt of a weak acid, and therefore swells less in acidic environment. The values of the settling volume of the samples in DW and SSF followed the ranking: NES < E3120M' < P3.5130M' < E3.5130M' < E3.5120M. The difference in settling volumes of the NES and the SCs is highly significant at p = 0.05.

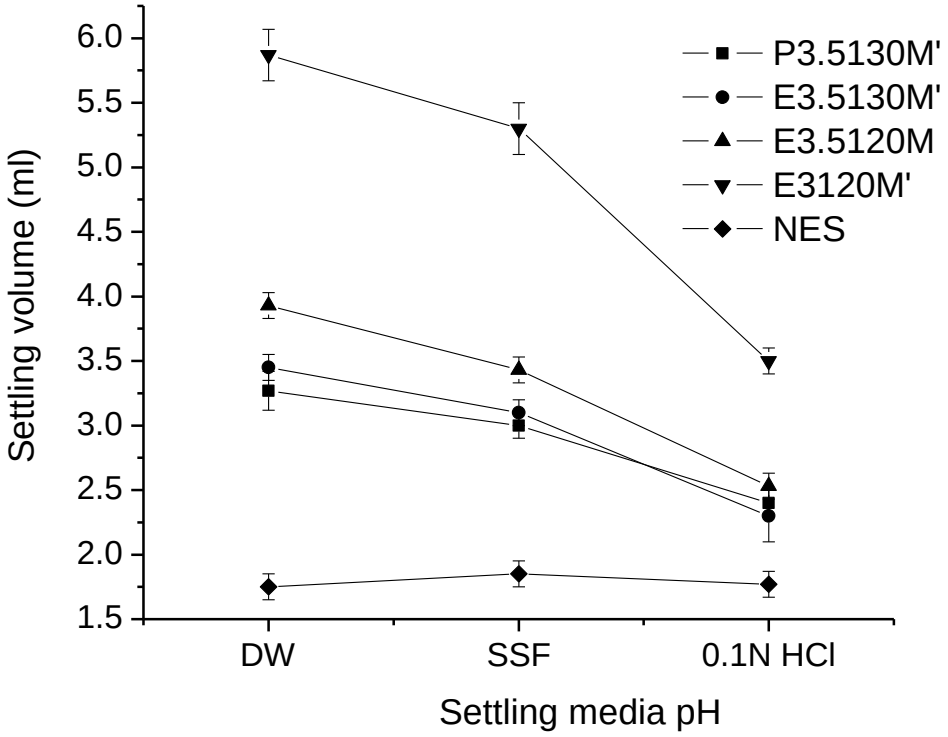

Figure 3.1: Settling volume of SCs and NES (0.5 mg in 25 ml) as a function of pH medium after 4 h. [P3.5130M': potato starch treated at pH 3.5, 130 ^{0}C and higher moisture content; E3.5130M': enset starch treated at pH 3.5, 130 ^{0}C and higher moisture content; E3.5120 M: enset starch treated at pH 3.5, 120 ^{0}C and lower moisture content; E3120M': enset starch treated at pH 3, 120 ^{0}C and higher moisture content; NES: native enset starch.]

The settling volumes were not affected by the presence of hydrophobic lubricants (Table 3.2). This observation is in contrast to expectations that the formation of a hydrophobic film around the particles might result in greater settling volume. This result may be due to incomplete film

formation of the lubricant around the disintegrant particles as a result of improper mixing or due to presence of soluble components in magnesium stearate. The difference in settling volumes between the concentrations of magnesium stearate is not statistically significant.

Table 3.2: Effect of magnesium stearate on settling volume of E3.5130M' SC in DW.

Sample composition	0% MgS	0.5% MgS	1% MgS	2% MgS	5% MgS
Settling Volume (ml)	3.45 ± 0.05	3.37 ± 0.15	3.33 ± 0.15	3.33± 0.15	3.30± 0.10

MgS: magnesium stearate

3.2.6 Swelling Power and Solubility

When starch is heated in excess water, the crystalline structure is disrupted and water molecules become linked by hydrogen bonding to the exposed hydroxyl groups of amylose and amylopectin. This causes an increase in granule swelling and solubility. Swelling power and solubility provide evidence of the magnitude of interaction between starch chains within the amorphous and crystalline domains (Okunlola and Akingbala, 2013). The extent of this interaction is influenced by the amylose/amylopectin ratio, and by the characteristics of amylose and amylopectin in terms of molecular weight/distribution, degree and length of branching, and conformation (Hoover, 2001).

Lee *et al*. (2005) showed that UV irradiation caused certain extensive associating forces that suppressed the swelling of granules, as well as solubility when compared to native starch ($p < 0.05$). This is believed to be the consequence of the strong aggregative forces in the granular organization. The bonding forces within the starch granules play an important role in determining their manner of swelling (Leach *et al.*, 1959).

The swelling power indicates indirectly the water holding capacity of native and modified starches (Singh and Nath, 2011).With the introduction of citrate, the granule structure may be altered. The citrate substituent may alter the associations between amylose and amylopectin and each component with itself in the granules. As can be observed from Figure 3.2, the difference in swelling power of the SCs and NES is highly significant. This may be because of the increase in hydrophilic functional groups as a result of modification. More water can penetrate into the starch granules due to the hydrophilicity of the carboxyl groups that result in swelling of the starch granules.

Figure 3.2 clearly shows that potato starch citrate is superior in swelling than ESC. This may be because the starch granules are slightly smaller thereby having greater surface area for reaction with citric acid. Amylose to amylopectin ratio, morphology and molecular weight can also be attributed to the better swelling of potato starch citrate. E3130M, E3120M and P3130M have lower swelling and the swelling is independent of temperature indicating that presence of moisture may be necessary for swellable SC to form. Among the ESCs, E3.5120M and E3.5130M had higher swelling, and the swelling is slightly constant throughout the range of temperature examined.

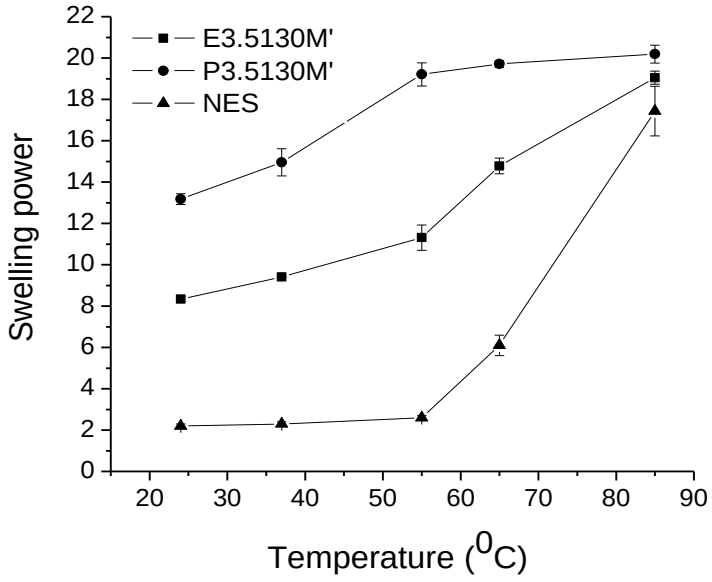

Figure 3.2: Swelling power of ESC, potato SC and NES. [P3.5130M': potato starch treated at pH 3.5, 130 ^{0}C and lower moisture content; E3.5130M': enset starch treated at pH 3.5, 130 ^{0}C and lower moisture content; NES: native enset starch.]

Citrate derivatives of cassava starch prepared by microwave having low degree of substitution were reported to possess lower swelling power at high temperature compared to the native starch (Jyothi *et al*., 2007). Xie and Liu (2004) have also reported that starch citrate resist granular swelling and absorb less water than the native starch. These results are in contrary to findings of the present study; and this may be due to the difference in modification parameters such as moisture content, temperature, starch source and duration of reaction. The method of swelling power determination may also be the reason for the difference.

Higher swelling indices were reported elsewhere (Chowdary *et al.*, 2011; Chowdary and Enturi, 2011a; Chowdary and Enturi, 2011b) whose main objectives were to modify for disintegrant products and their results were in line with the findings of the present study.

Similar swelling power profiles had been reported for tacca starch and its citrate modification at all temperature of examination (20 - 100 ^{0}C) (Adebiyi *et al.*, 2011).

Most of the SCs prepared have constant SP which shows that there is certain point beyond which the swelling of the SCs is independent of temperature of swelling determination. At lower temperature, the swelling of the native starch is significantly lower than the SCs. The results of this study suggest that most of the modification resist increment in granular swelling with temperature while others with a different set of reaction conditions increase swelling to a certain extent. All type of starch, pH of medium, temperature of modification and moisture content affect the results.

Figure 3.3 depicts the solubility profile of the SC and NES. Almost all of the SCs have solubility higher than the NES at lower temperatures. This may be due to the incorporation of the citrate to smaller granules of the starch which increases their solubility. The increase in solubility after citrate modification can also be as a result of depolymerization and structural weakening of the starch granules. This may be a disadvantage for the SCs to use as disintegrant because poor solubility is an ideal character for a disintegrant.

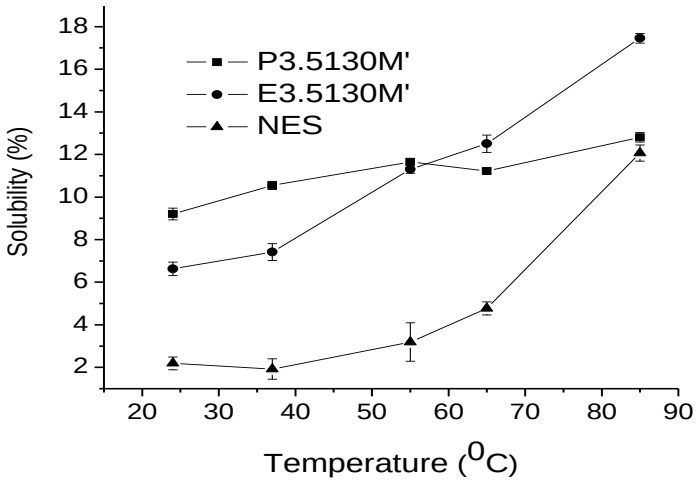

Figure 3.3: Solubility of the SCs prepared at different reaction conditions, and NES
[P3.5130M': potato starch treated at pH 3.5, 130 ^{0}C and lower moisture content; E3.5130M': enset starch treated at pH 3.5, 130 ^{0}C and lower moisture content; NES: native enset starch.]

Figure 3.4 (A) - (F) compares the difference in the swelling power of the ESCs at different reaction parameters at one constant variable. Figure 3.4 (A) and (B) show the influences of the other factors on swelling at 120 and 130 ^{0}C respectively. Figure 3.4 (C) and (D) show the

influences of the other factors on swelling at pH 3 and 3.5 respectively. Figure 3.4 (E and F) show the influences of the other factors on swelling at low and high moisture levels respectively.

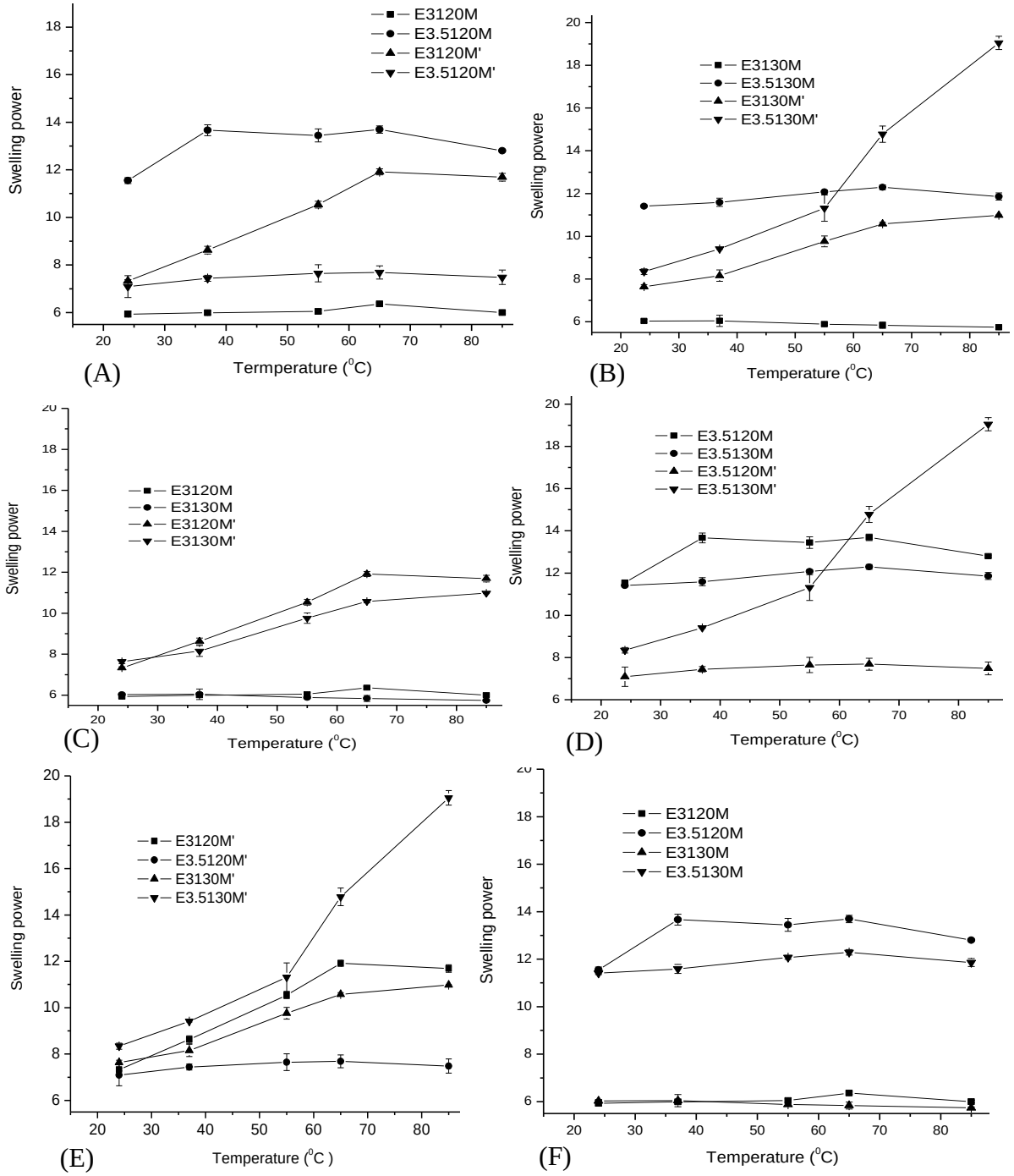

Figure 3.4: Comparison of swelling power at different modification parameters: (A) effect of pH and moisture at 120 ^{0}C; (B) effect of pH and moisture at 130 ^{0}C; (C) effect of moisture and temperature at pH 3; (D) effect of moisture and temperature at pH 3.5; (E) effect of temperature and pH at low moisture content and (F) effect of temperature and pH at relatively moderate moisture content.

3.2.7 Screening of SCs for Disintegrant Effect

Table 3.3 compares physical characteristics of ibuprofen tablets for direct compression formulation employing ESCs as disintegrant. Although the swelling power of E3.5130M and E3.5120M is higher than that of E3.5130M' and E3120M' at 37 ^{0}C, DT is higher for tablets which contain E3.5130M and E3.5120M. This may indicate that swelling may not be the predominant mechanism of disintegration of the SCs. Although the disintegration mechanism is not fully understood and no general conclusions can be drawn from the contradictory results presented, there is no doubt that water uptake must be the first step in any process of disintegration.

Table 3.3: Screening of the SCs at 3% level as disintegrant for DT in ibuprofen tablets.

Attribute	E3.5130M	E3.5130M'	E3120M'	E3.5120M
Weight (mg)	380.1±1.81	381.4±0.52	377.4±1.78	379.8±2.09
Hardness (N)	118.7±13.32	114.7±5.0	110.9±11.5	120.3±7.63
DT (min)	1.4±0.09	0.83±0.01	0.85±0.02	1.06±0.23

Among these SCs in Table 3.3, E3.5130M' is a better disintegrant and was selected for further tableting analysis.

3.2.8 Moisture Sorption Capacity

Active pharmaceutical ingredients (APIs) as well as excipients in a solid dosage form can take up water vapor both during manufacture and subsequent storage of the product. Uptake of unacceptable amount of water can cause adverse effects on physical and chemical stability of APIs and functionality of excipients. It is prudent to select drug candidates with low hygroscopicity to minimize the development risk and time.

Water in pharmaceutical products either as the residual water from processing or as the result of exposure to high relative humidity (RH) may affect the chemical or physical stability of moisture-sensitive products (Nanjwade *et al.,* 2010). Moisture transfer from gelatin capsule shells to the hygroscopic fill contents or to low RH environment may result in brittle capsule products (Kontny and Mulski, 1989). Sorption of moisture from the manufacturing environment by hygroscopic granules can lead to a sticking problem during compression. Equilibrium moisture sorption isotherm of a product is a key factor governing the rate of moisture uptake by the packaged product during shelf life, besides the environmental

conditions and container permeability (Kontny *et al*., 1992). Therefore, it is beneficial if the moisture sorption isotherm of the excipient can be predicted at the early stage of formulation development. Such a prediction can guide the excipient selection in formulation design to increase the efficiency of product development (Yanxia *et al*., 2003).

During the modification hydrophilic carboxyl groups are introduced to the starch granules. It can be anticipated that the resulting product be more hydrophilic than the native starch. Although starch is hygroscopic the SCs are more hygroscopic than it, so great care should be taken in handling and processing. The higher capacity of starch to absorb water was indicated to facilitate disintegration (Jubril *et al*., 2012). Profiles of moisture sorption and swelling power of this study indicate that starch citrate is promising disintegrant in tablet formulations. Figure 3.5 displays the moisture sorption in percent weight by weight as a function of percent RH at room temperature. The moisture sorption of the samples at same RH values shows that there is significant difference ($p < 0.05$) in sorption between the samples.

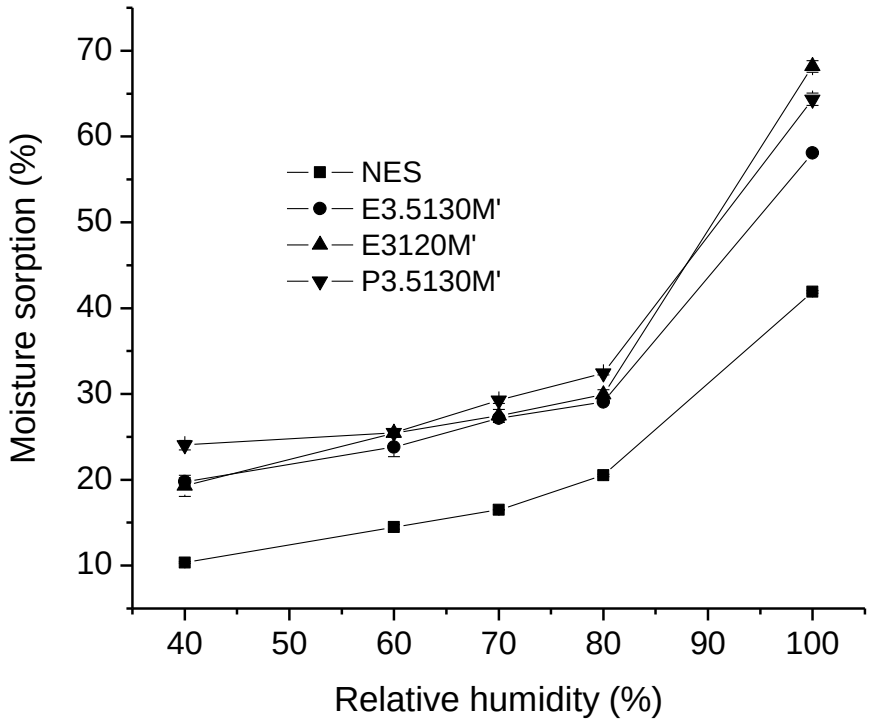

Figure 3.5: Moisture sorption profiles of SCs and NES from 40% to 100% RH at room temperature. [P3.5130M': potato starch treated at pH 3.5, 130 ^{0}C and lower moisture content; E3.5130M': enset starch treated at pH 3.5, 130 ^{0}C and lower moisture content; E3120M': enset starch treated at pH 3, 120 ^{0}C and lower moisture content; NES: native enset starch.]

For the samples at 40% RH, means comparison using 'bonferroni test' shows, E3.5130M' has no significant difference in sorption compared to E3120M' and P3.5130M' but differs significantly in sorption compared to the NES; E3120M'has significant difference in sorption compared to P3.5130M' & the NES; and P3.5130M' differs significantly in sorption from the NES. Similarly, at 60% RH E3.5130M' has no significant difference in sorption compared to E3120M' and P3.5130M' but has significant difference in sorption compared to the NES; E3120M' differ significantly in sorption compared to the NES but has no significant difference in sorption compared to P3.5130M'; and P3.5130M' has significant difference in sorption compared to the NES. Same result is obtained at 70% RH as that found in 60% RH. At 80% RH all differ significantly in sorption except E3.5130M' and E3120M'. No significant difference is observed between E3.5130M', E3120M' and P3.5130M', but all differ significantly from the NES at 100% RH.

3.2.9 Morphological Characterization

Particle morphology is an important property in the characterization and identification of, especially powdered pharmaceutical excipients. It can also be used to predict certain functional properties that relate particularly to flowability and compactibility of the powder and disintegrating characteristics of tablets (Builders *et al.*, 2013).

The scanning electron micrographs of the NES and an SC are depicted on Figure 3.6. As can be seen from Figure 3.6, the granules of the NES have characteristic morphology- angular and elliptical in shape, and exist as a single entity. The surface of the granules is smooth, but some exhibit fissure (cracks) when viewed at larger magnifications. These cracks or damage could be due to aging of the plant during starch isolation. However, granule shapes changed when starches were treated with citric acid (Figure 3.6 (C) and (D)). The center of the granules appears swollen than their outer parts.

Xie *et al.* (2006) reported the morphology of maize starch citrate granules as doughnut-shaped with their outer sides drawn inwards; this is in contrary to the results of this study: the particles of the SCs are ruptured and have no definite shape and are somewhat feathery. The morphology of tacca starch and its citrate has been examined to look alike but some granules in the starch citrate appear ruptured (Adebiyi *et al*., 2011); same result was reported for icacina starch and its modification (Omojola *et al.,* 2012). This may be attributed to the differences in set of reaction conditions or to type of starches.

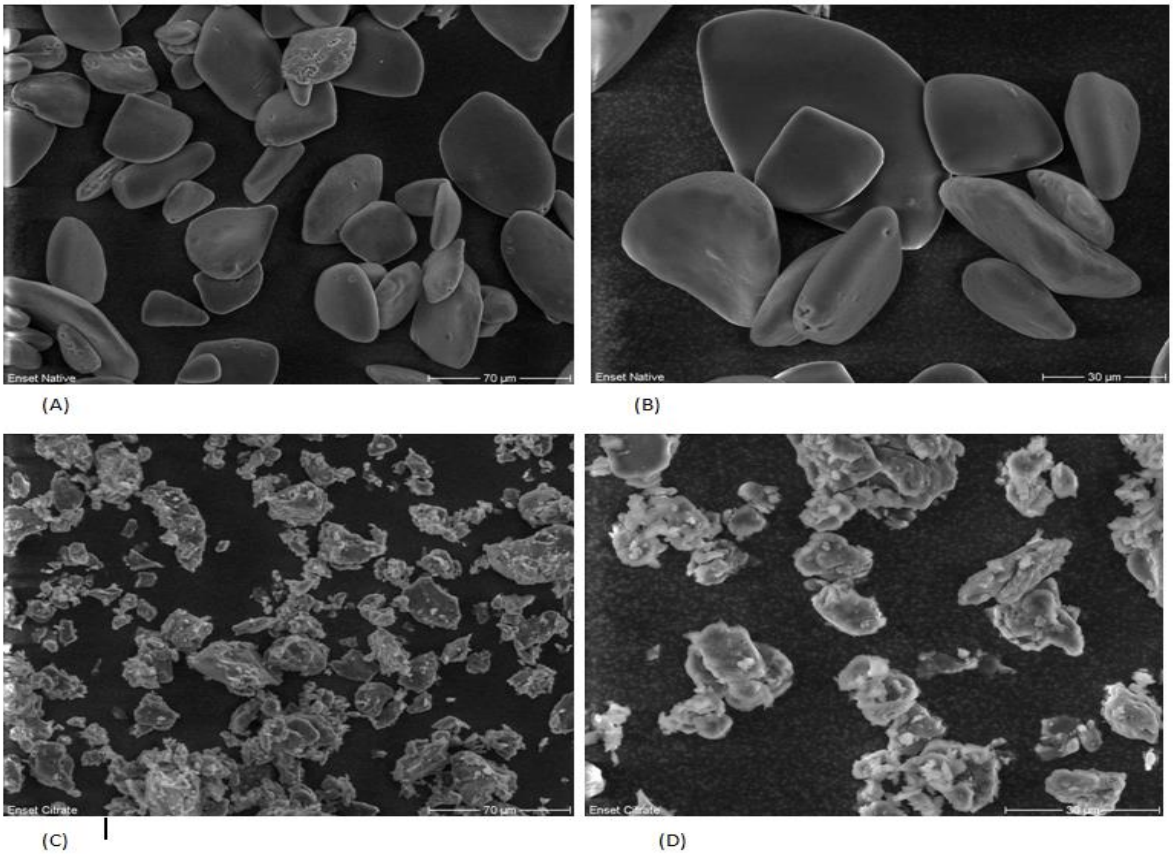

Figure 3.6: Scanning electron micrographs of native enset starch granules: (A) lower magnification (70 µm scale bar) and (B) higher magnification (30 µm scale bar); and enset starch citrate: (C) lower magnification (70 µm scale bar) and (D) higher magnification (30 µm scale bar)

SEM (Figure 3.6 (C) and Figure 3.6 (D)) suggest that chemical reagents such as citric acid could directly access a loosely organized region in the center of starch by channels and cavities, which might lead to an alteration in the granule morphology. Figure 3.6 (C) and (D) also show that the granule surfaces of starch citrates were less smooth than those of their natives.

3.2.10 Determination of Flow Properties of SCs

During product manufacture, large volumes of powder blends are fed through production equipment/processes, and it is essential to be able to accurately determine, define, and control powder properties to ensure reproducible manufacture and product performance. Therefore the characterization of the physicochemical properties of powder blends is extremely important (BP, 2009).

Four commonly reported methods for testing powder flow are: angle of repose, compressibility index of Hausner's ratio, flow rate through orifice and shear cell. No one simple powder flow method will adequately or completely characterize the wide range of flow properties. An appropriate strategy is the use of multiple standardized test methods to characterize the various aspects of powder flow (JP, 2006).

3.2.10.1 Determination of Density and Related Properties

In determining true density, it is assumed that the system is homogeneous with no intra-particulate void. True density of pharmaceutical crystals is typically between 1 and 2.5 g/ml. True density**,** bulk density, tapped density, compressibility index and Hausner's ratio are given in Table 3.4. The true density of E3.5130M' is lower than that of the NES.

The bulk density of a powder is not a definite number like true density or specific gravity but an indirect measurement of a number of factors, including particle size and size distribution, particle shape, true density, and especially the method of measurement. Although there is no direct linear relationship between the flowability of a powder and its bulk density, the latter is extremely important in determining the capacity of mixers and hoppers and providing an easily obtained valuable characterization of powders. The bulk density for the samples examined followed the ranking: E3120M' < NES < E3.5130M' < P3.5130M' (Table 3.4)

Scale of flowability with compressibility index and Hausner's ratio is given elsewhere (JP, 2006). The results for compressibility index and Hausner's ratio show that the powders range from good to poorly flowable. P3.5130M', NES, E3120M' and E3.5130M' have good, fair, passable and poor flow, respectively, based on Carr's index and Hausner's ratio.

3.2.10.2 Angle of Repose

A material that is not cohesive flows well, spreads out, forming a low heap. More cohesive materials form higher heaps. The angle of repose is defined as the angle of the free surface of a pile of powder to the horizontal plane. The angle of repose of NES, P3.5130M', E3.5130M' and E3120M' is given in Table 3.4. As can be seen from the table, the samples range from good to passable in flowability based on angle of repose (JP, 2006).

Table 3.4: Summary of flow properties of NES and SCs prepared from enset and potato starches at different reaction conditions.

Sample	True density (g/cc)	Bulk density (g/cc)	Tapped density (g/cc)	Compressibility index (%)	Hausner's ratio	Angle of repose (^{0})
NES	1.47±0.01	0.62±0.01	0.75±0.00	18±0.6	1.21±0.004	34±0.9
P3.5130M'		0.67±0.01	0.78±0.01	14±0.2	1.17±0.02	35±1.6
E3.5130M'	1.26±0.01	0.63±0.01	0.85±0.01	26±0.5	1.36±0.01	39±2.3
E3120M'		0.59±0.00	0.78±0.00	24±0.3	1.32±0.01	40±0.3

3.3 DSC Study of Drug-excipient Compatibility

The study of drug-excipient compatibility is an important process in the development of a stable solid dosage form. Interactions between drug and excipient can give rise to changes in bioavailability and stability, which in turn affect safety and/or efficacy of the drug. Some changes or modifications in shape, peak temperature or area may arise simply from mixing of the components in drug-excipient compatibility experiments by DSC. Appearance of new peaks or disappearance of ibuprofen peaks or major shift in the peak temperature were major criteria in determining incompatibility of excipients with drug (Late and Banga, 2008). The result is depicted in Figure 3.7. It can be concluded from the figure that no interaction exists between ibuprofen and the ESC.

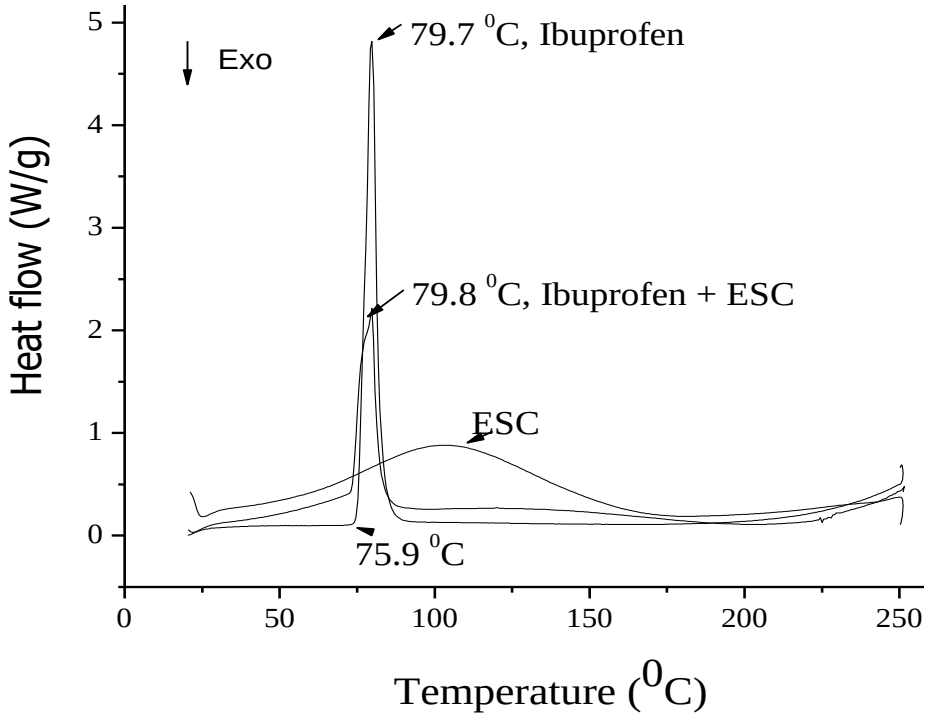

Figure 3.7: DSC curves of ibuprofen, ESC and 1:1 mixture of ibuprofen and ESC.

The DSC thermograms of a SC gelatinization were studied by Xie and Liu (2004). Compared to their controls (native starch with same treatment except citric acid), the SCs exhibited no gelatinization peak. The peak in Figure 3.7 for ESC may be that of water residue in it.

3.4 Tablet Formulation and Tableting by Direct Compression

3.4.1 Preparation of Powder Mixture

3.4.1.1 Determination of Density and Related Properties, and Angle of Repose

Table 3.5 shows the bulk density, tapped density, Carr's index, Hausner's ratio and angle of repose of the blends for screening for the formulation of ibuprofen tablets. The bulk density of the four formulations ranges from 0.400 to 0.414 g/cc. The powders range from 0.474 g/cc to 0.501 g/cc in tapped density; from 14 to 19 in Carr's index; from 1.22 to 1.23 in Hausner's ratio and from 34^0 to 36^0 in angle of repose.

With reference to JP 15^{th} edition specifications, Carr's index and Hausner's ratio of powder mixture C has good flow character while A, B and D have fair flow with respect to Carr's index and Hausner's ratio (Table 3.5). Analysis of angle of repose shows formulation B and C have good flow but A and D have fair flow.

Table 3.5: Flow properties of the powder blend for ibuprofen tablets.

Formulations	Bulk density (g/cc)	Tapped density (g/cc)	Carr's index (%)	Hausner's ratio	Angle of repose (degree)
A	0.407±0.005	0.501±0.006	19	1.23	36±1.5
B	0.405±0.003	0.495±0.006	18	1.22	34±1.2
C	0.414±0.003	0.483±0.002	14	1.17	35±0.7
D	0.400±0.008	0.474±0.002	16	1.19	36±0.6

3.4.2 Compression of Powder into Tablet

Table 3.6 gives tablet characteristics for the screening of directly compressed formulation of ibuprofen tablets at 3% SC concentration. Tablet analysis in Table 3.6 shows formulation A has lowest DT but formulation C has longest DT. Based on these data, formulation A was taken for further evaluation of the influence of CF and DisC on tablet characteristics in ibuprofen tablets for the optimization formulation. After several preliminary tests, the minimum and maximum percent of ESC is selected to be 2% & 4%. Optimization was

performed using three-level factorial design in response surface method. Compression of the formulations was conducted at 4 KN, 7 KN and 10 KN CFs.

Table 3.6: Tablet characteristics for the screening of ESC at 3% in ibuprofen tablets.

Parameter	Formulations			
	A	B	C	D
Wt (mg)	377 (5)	393 (4)	375 (2)	396 (5)
DT(sec.)	55 (1)	166 (6)	205 (14)	154 (23)
Hardness (N)	99 (10)	115 (18)	114 (7)	96 (21)
Friability (%)	0.48	0.46	0.48	0.47

The values in parenthesis indicate the SD.

3.5 Comparison of ESC and SSG as Disintegrants

The effect of CFs and levels of ESC and SSG on DT, hardness and friability is given in Table 3.7. As can be clearly seen from Table 3.7, the difference in hardness between those tablets containing SSG and ESC is statistically significant ($p < 0.05$) at 10 KN CFs; when CF increases hardness increases for the ESC containing tablets. The higher hardness of those tablets containing ESC may show that it may have binding effect. Although the trend is erratic, higher hardness of tablets can be achieved with lower CFs with ESC containing tablets. DT values as low as 25 sec can be obtained at 4% ESC levels at well above 60 N hardness. At 4% ESC levels hardness as strong as 151 N can be produced at 10 KN CF. In all the CFs examined for SSG containing tablets, the values for DT are observed to be less than one min demonstrating the superiority of SSG as disintegrant. Tablets with SSG are more friable than those with ESC.

Table 3.7: Effect of CF and ESC/SSG levels on DT (sec), hardness (H (N)) and friability (F, %) of ibuprofen tablets.

Type	CF (KN)	2%			3%			4%		
		H	F	DT	H	F	DT	H	F	DT
ESC	4	63 (4)	0.15	55 (2)	67 (3)	0.16	51 (2)	65 (3)	0.17	25 (1)
	7	109 (3)	0.11	105 (2)	124 (7)	0.1	116 (1)	110 (4)	0.11	50 (1)
	10	139 (5)	0.14	187 (5)	145 (7)	0.12	157 (2)	151 (5)	0.1	95 (2)
SSG	7	102 (5)	0.14	31 (1)	108 (5)	0.17	32 (1)	100 (4)	0.17	33 (1)
	10	118 (7)	0.13	42 (3)	109 (12)	0.15	39 (1)	115 (7)	0.16	41 (2)
	15	114 (8)	0.13	47 (3)	127 (9)	0.15	45 (7)	126 (8)	0.12	44 (2)
	20	122 (10)	0.15	52 (7)	128 (8)	0.16	63 (2)	132 (8)	capped	46 (2)

3.6 Optimization Study

DoE makes controlled changes to input variables in order to gain maximum amounts of information on cause and effect relationships with a minimum sample size. DoE is more efficient than a standard approach of changing "one variable at a time" in order to observe the variable's impact on a given response (Jaimini *et al.*, 2013). DoE can be used to identify critical variables and ultimately create mathematical models of the process being examined aimed at predicting process performance (Van Buskirk *et al.*, 2014).

The dissolution of poorly soluble drugs in tablets can be enhanced by lowering the time the drug particles come in contact with the dissolution medium by including effective disintegrants. Therefore, the appropriate type and amount of disintegrant is in many cases the critical factor determining tablet quality. Tablets also require hardness and resistance to friability to withstand mechanical shocks of handling in manufacture, packaging, and shipping. Therefore this study optimizes the DisC and CF in ibuprofen tablets.

Preliminary experiments undertaken to determine the range of DisC and CF for the compression of the formulations of ibuprofen tablets show that DisC of 2% and 4%, and CF of 4 KN and 10 KN as the maximum and minimum values (Table 2.3).

Second-order models are widely used in RSM as they have several advantages (Murphya et al., 2004; Bezerra et al., 2008; Ebrahimi et al., 2010). Replicate observations at the center point were used to estimate the experimental error employed (Aktas, 2005). The complete set of experiments was performed in a random order, to avoid systematic error. Table 3.8 shows the experimental results for the responses of DT, hardness and friability obtained from the 11 formulations produced as per the designed plan. These results were fed into the Design Expert® V 9.0.6.2 software for the optimization analysis.

Table 3.8: Summary of experimental response values of the 11 formulations of ibuprofen tablets produced by direct compression method.

Run	Responses		
	DT (sec)	Hardness (N)	Friability (%)
F_1	186.7	138.5	0.14
F_2	119.3	116.4	0.10
F_3	51.0	66.5	0.16
F_4	148.3	116.7	0.09
F_5	25.3	65.2	0.17
F_6	137.0	114.1	0.11
F_7	50.3	110.3	0.11
F_8	95.3	151.0	0.10
F_9	54.7	62.7	0.15
F_{10}	157.3	125.4	0.12
F_{11}	105.3	109.2	0.11

3.6.1 Mathematical Model Selection

Fit summaries are generated using the Design Expert® software to obtain suitable models for the responses. Table 3.9 gives the fit summaries output of the responses. It gives statistics such as p-value, the multiple correlation coefficient (R^2), adjusted (Adj) R^2, predicted (Pred) R^2 and the predicted residual sum of square (PRESS) values for comparing linear, interaction and quadratic models for each response. The program selects and suggests for further use the highest order polynomial where the additional terms are significant and the model is not aliased, the Adj R^2 and $Pred^2$ are in a reasonable agreement (within 0.20 of each other) and the model maximizing the Adj R^2 and the $Pred^2$. A model is considered significant if its p-value is less than 0.05 or at least less than 0.1. A model with small PRESS value is desirable. PRESS

is a measure of how well the model for the experiment is likely to predict the responses in a new experiment. According to the fit summary output, linear model and quadratic models were suggested and selected as best fit models for hardness, and DT and friability respectively.

R^2 is the proportion of variation in the response attributed to the model rather than to random error and was suggested that for good fit model, R^2 should be at least 80% (Gan *et al.*, 2007). The results of this study showed that the models for all the responses were highly adequate because they have satisfactory levels of R^2 of more than 80%.

Table 3.9 shows that R^2 for the chosen models is high (> 0.9) for all responses, which indicates a high degree of correlation between the experimental and predicted responses. Besides, the chosen models for all the responses are the models that maximize the Pred R^2 and Adj R^2 values which indicate reliable models.

Table 3.9: Fit summary statistics for DT (Y_1 (sec)), hardness (Y_2, (N)) and friability (Y_3, (%)) for the ibuprofen tablets.

Responses	Source	Std. Dev.	R^2	Adj R^2	Pred R^2	p-value Prob > F	PRESS	Remark
Y_1	Linear	28.18	0.7677	0.7097	0.5873	0.0029	11284.48	
	2FI	27.75	0.8029	0.7184	0.5507	0.3008	12286.26	
	Quadratic	18.09	0.9401	0.8803	0.6712	0.0508	8990.81	Suggested
	Cubic	23.22	0.9408	0.8028	-4.5607	0.9826	$1.52*10^5$	Aliased
Y_2	Linear	9.48	0.9189	0.8986	0.8543	< 0.0001	1291.67	Suggested
	2FI	9.96	0.9217	0.8882	0.8021	0.6309	1754.80	
	Quadratic	7.85	0.9652	0.9305	0.6925	0.1316	2726.55	
	Cubic	6.70	0.9848	0.9493	-0.8743	0.2889	16616.94	Aliased
Y_3	Linear	0.024	0.3400	0.1750	-0.3696	0.1897	$9.94*10^{-3}$	
	2FI	0.024	0.4641	0.2344	-0.9511	0.2437	0.014	
	Quadratic	$7.70*10^{-3}$	0.9591	0.9183	0.8420	0.0016	$1.15*10^{-3}$	Suggested
	Cubic	$9.37*10^{-3}$	0.9637	0.8791	-0.1569	0.8362	$8.39*10^{-3}$	Aliased

3.6.2 Model Adequacy Checking

The adequacy of the model was evaluated by model p-value at the 0.05 significance. The statistical analysis of RSM for the responses was performed by using Design Expert® (V 9.0.6.2; Stat-Ease Inc., Minneapolis, MN, USA) software. ANOVA was used as a tool to evaluate significance and goodness of fit of the regressional model and significance of individual model coefficients (Murphya *et al.*, 2004; Gan *et al.*, 2007). The ANOVA report includes an indication as to whether or not the selected model is statistically significant or not at the default confidence level of 95%.

Table 3.10 shows the quadratic model of DT and friability, and linear model of hardness are statistically significant ($p < 0.05$) which is desirable as it demonstrates the terms in the model have significant effect on the responses. The software suggests that if there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve the model. For DT the terms AB (interaction effect of CF and DisC) and A^2 (second order effect of CF) are not significant model terms whereas the terms A (CF), B (DisC) and B^2 (second order effect of DisC) are significant model terms. For hardness the term A is significant model term while B is insignificant. For friability, the terms A and B^2 are significant model terms while B (is in the hierarchy) and A^2 are insignificant model terms. To increase the predictive efficiency of the models, backward elimination procedure was applied to reduce insignificant terms.

Table 3.10: ANOVA table for response surface linear model of hardness and quadratic model of DT and friability for the directly compressed formulation of ibuprofen tablets.

Responses	Source	Sum of Squares	Df	Mean Square	F-Value	p-value Prob > F	
Y_1	Model	25706.50	5	5141.30	15.70	0.0045	Significant
	A- CF	15841.48	1	15841.48	48.39	0.0009	
	B-DisC	5150.94	1	5150.94	15.73	0.0107	
	AB	961.00	1	961.00	2.94	0.1473	
	A^2	156.74	1	156.74	0.48	0.5198	
	B^2	2965.82	1	2965.82	9.06	0.0298	
	Lack of Fit	1209.61	3	403.20	1.89	0.3648	Insignificant
Y_2	Model	8146.58	2	4073.29	45.33	< 0.0001	Significant
	A- CF	8103.38	1	8103.38	90.18	< 0.0001	
	B-DisC	43.20	1	43.20	0.48	0.5077	
	Lack of Fit	714.86	6	119.14	59.41	0.0166	Significant
Y_3	Model	$6.96*10^{-3}$	5	$1.39*10^{-3}$	23.47	0.0018	Significant
	A- CF	$2.4*10^{-3}$	1	$2.4*10^{-3}$	40.47	0.0014	
	B-DisC	$6.67*10^{-5}$	1	$6.67*10^{-5}$	1.12	0.3375	
	AB	$9.0*10^{-4}$	1	$9.0*10^{-4}$	15.18	0.0115	
	A^2	$3.06*10^{-3}$	1	$3.06*10^{-3}$	51.55	0.0008	
	B^2	$5.68*10^{-5}$	1	$5.68*10^{-5}$	0.96	0.3725	
	Lack of Fit	$9.65*10^{-5}$	3	$3.22*10^{-5}$	0.32	0.8143	Insignificant

Table 3.11 shows the ANOVA results for response surface reduced quadratic models of DT and friability and reduced linear model of hardness of the directly compressed formulation of ibuprofen tablets. The results indicate that the reduced models are more significant than the original models indicating the model's predictive efficiency has been improved. For DT, hardness and friability, the F-values and p-values for the reduced and unreduced models are respectively $F = 20.7$, $p < 0.0007$, $F = 15.7$, $p < 0.0045$; $F = 95.72$, $p < 0.0001$, $F = 45.33$, $p < 0.0001$; and $F = 37.97$, $p < 0.0001$, $F = 23.47$, $p < 0.0018$.

Table 3.11: ANOVA table for response surface reduced quadratic models of DT and friability and reduced linear model of hardness of directly compressed formulation of ibuprofen tablets.

Response	Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	Remark
Y_1	Model	24588.76	3	8196.25	20.83	0.0007	significant
	A-CF	15841.48	1	15841.48	40.26	0.0004	
	B-DC	5150.94	1	5150.94	13.09	0.0085	
	B^2	3596.34	1	3596.34	9.14	0.0193	
	Lack of Fit	2327.35	5	465.47	2.18	0.3439	insignificant
Y_2	Model	8103.38	1	8103.38	95.72	< 0.0001	significant
	A-CF	8103.37	1	8103.37	95.72	< 0.0001	
	Lack of Fit	757.87	7	108.27	53.51	0.0185	significant
Y_3	Model	$6.84*10^{-3}$	3	$2.28*10^{-3}$	37.97	0.0001	significant
	A-CF	$2.4*10^{-3}$	1	$2.4*10^{-3}$	40.00	0.0004	
	B-DisC	$6.67*10^{-5}$	1	$6.67*10^{-5}$	1.13	0.3283	
	AB	$9.0*10^{-4}$	1	$9.0*10^{-4}$	15.00	0.0061	
	A^2	$3.54*10^{-3}$	1	$3.54*10^{-3}$	58.91	0.0001	
	Lack of Fit	$2.2*10^{-4}$	5	$4.4*10^{-5}$	0.44	0.8014	insignificant

The lack of fit (LOF) test is a measure of the failure of a model to represent data in the experimental domain at which points were not included in the regression (Varnalis *et al.*, 2004). For a model to be successfully used for prediction, the LOF should be 'not significant' (Sahoo *et al.*, 2011; Sahoo and Gupta, 2012). The p-values of LOF (0.3439 and 0.8014 for Y_1, and Y_3, respectively) were greater than 0.05, that strengthens the models are reliable. Although the p-value of LOF for Y_2 (0.0185) is significant which is bad, the "Pred R^2" of 0.8614 is in reasonable agreement with the "Adj R^2" of 0.9045, and has an "Adeq precision" of 18.734 (Table 3.12), which measures the signal to noise ratio, indicate an adequate signal to imply that this model can be used to navigate the design space.

Diagnostic plots are provided by the Design-Expert® software to test if a model is adequate to describe the observed data (Bashir *et al.*, 2010). The normal probability plot indicates whether the residuals follow a normal distribution, in which case the points will follow a straight line.

Table 3.12: Summary of statistical test results of model adequacy checking for the reduced linear model of Y_2 and reduced quadratic model of Y_1 and Y_3.

Statistics	Y_1	Y_2	Y_3
PRESS	7577.86	1228.58	$8.0*10^{-4}$
R^2	0.8993	0.9141	0.9513
Adj R^2	0.8561	0.9045	0.9188
Pred R^2	0.7229	0.8614	0.8897
Adeq precision	14.076	18.734	13.723

Adeq: Adequate

Figure 3.8 (A) - (F) depict normal plot of residuals ((A), (C) and (E)) and residuals VS predicted ((B), (D) and (F)) diagnostic plots for Y_1, Y_2, and Y_3, respectively, for the formulations of ibuprofen tablets. As can be seen from Figure 3.8 (A), (C) and (E), definite pattern of "S-shaped" curve is not available for all the responses which indicates the models are adequate to describe the observed data.

The residuals VS predicted plot is a plot of the residuals versus the ascending predicted response values. It tests the assumption of constant variance. The plot should be a random scatter (constant range of residuals across the graph). Expanding variance in this plot indicates that the model doesn't adequately represent the data. This plot also helps to detect outliers in the data. Plots of Figure 3.8 (B), (D) and (F) show that the residuals of Y_1, Y_2 and Y_3 are scattered randomly and that there are no outliers, data points that are not fit well by the current model.

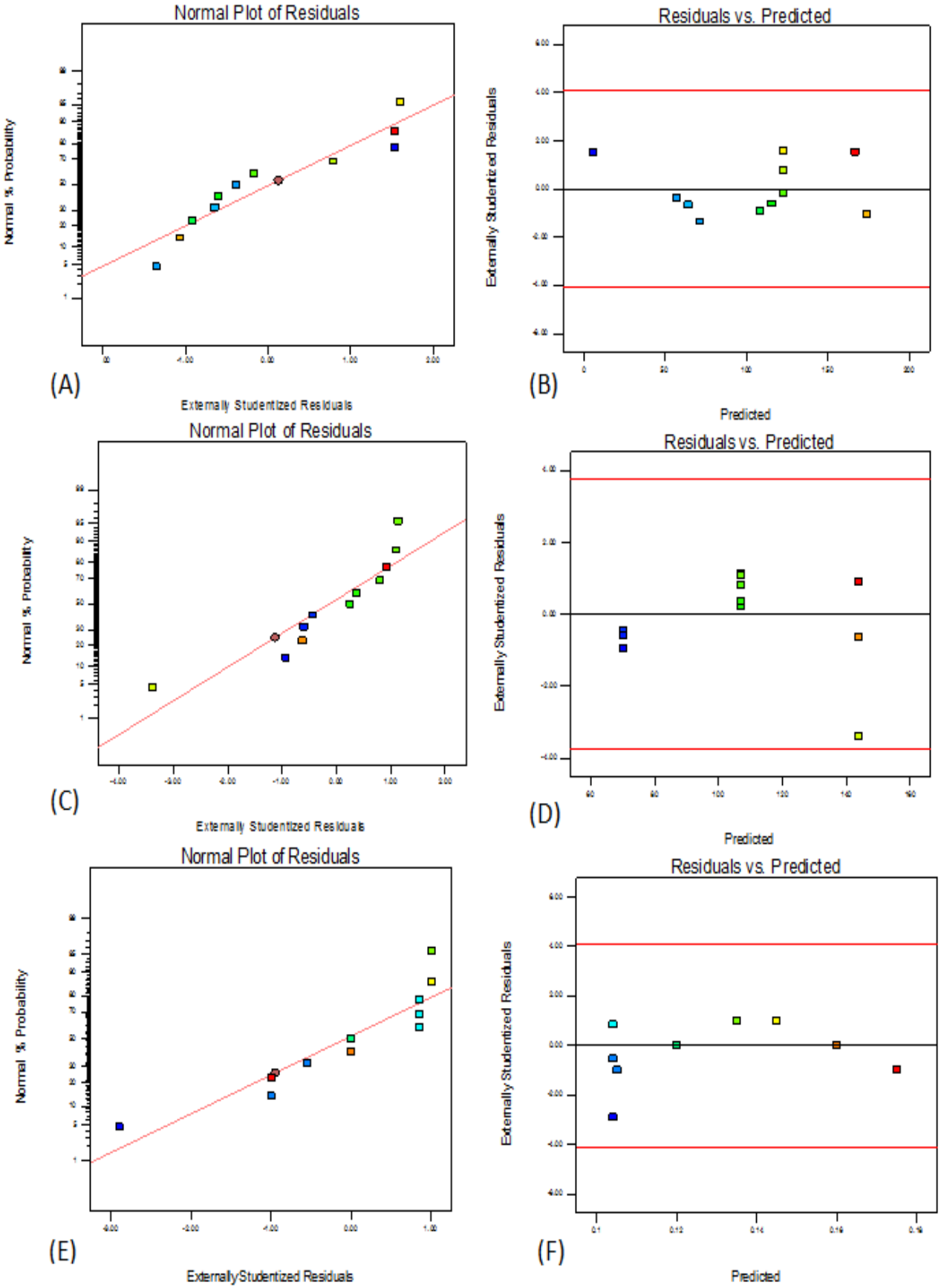

Figure 3.8: Normal plot of residuals and residuals VS. predicted diagnostic plots for Y_1((A) and (B)), Y_2 ((C) and (D)), and Y_3 ((E) and (F)), respectively, of ibuprofen tablets.

Therefore, predictive mathematical models were established for the responses based on the various levels of the factors. The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients. After the regression coefficients were obtained, the estimated response could be easily calculated using the model equation (Boyaci, 2007). The final equations in terms of coded factors are given from Eq. 3.1 to Eq. 3.3:

$$Y_1 = 122.58 + 51.38 * A - 29.3 * B - 36.31 * B^2 \quad (3.1)$$

$$Y_2 = 106.91 + 36.75 * A \quad (3.2)$$

$$Y_3 = 0.10 - 0.020 * A - 3.33 * 10^{-2} * B - 0.015 * AB + 0.036 * A^2 \quad (3.3)$$

where A is CF and B is DisC

Coefficients with more than one factor term and those with higher order terms represent interaction terms and quadratic relationships respectively. A positive sign represents a synergistic effect, while a negative sign indicates an antagonistic effect on the responses (Nazzal and Khan, 2002). Eq. 3.1 shows that CF has the largest influence on the DT, and has positive effect on it. This may be because ibuprofen particles are more cohesive and sticky. The quadratic term of B has antagonistic effect on Y_1.

3.6.3 Model Graphs: Contour and 3D Surface Plots for Y_1, Y_2 and Y_3.

Contour and 3D surface plots of the desirability function at each optimum can be used to explore the function in the factor space. Any individual response may be graphed to show the optimum point. The visualization of the predicted model equation can be obtained by the response surface plot and contour plot. The response surface plot is the theoretical three-dimensional plot showing the relationship between the response and the factors. The two-dimensional display of the surface plot is called contour plot and in the contour plot, lines of constant response are drawn in the plane of the independent variables. The contour plots help to visualize the shape of a response surface. When the contour plot displays ellipses or circles, the center of the system refers to a point of maximum or minimum response (Boyaci, 2007).

Contours are curves of constant response drawn in the xi, xj plane keeping all other variables fixed. Each contour corresponds to a particular height of the response surface, as shown in

Figure 3.9 (A), (C) and (E). These types of plots show the effects of two factors on the response at a time. It may be clearly understood from the variety of colors the influence of the factors on the responses – graduated from cool blue for lower response levels to warm yellow for higher values on the contour and 3D surface plots.

Figure 3.9 (A) and (B) show the contour and 3D surface plots for Y_1 of ibuprofen tablets. The slightly curved lines of the contours indicate that there is interaction effect of the factors CF and DisC on the DT of the formulations, but the interaction is not significant as elliptical contours are not available. The slight curvature of the contours and response surfaces, and the ANOVA results in Table 3.10 ($p = 0.1473$) indicate that the interaction effect of the two variables is not significant. The colors of the contour and the 3D surface also clearly show that at lower CF and higher DisC the response is low and the response is low at high CF and medium DisC as can be evidenced by the cool blue and warm yellow to red color of the plots. At lower CFs, lower DT values are obtained at higher DisC.

Figure 3.9 (C) and (D) show the contour and 3D surface plots for Y_2 of the ibuprofen tablets. The straight lines of the contour plot and the flat surfaces of the 3D surface indicate that there is no interaction effect of the factors CF with DisC on the hardness of the formulations.

Figure 3.9 (E) and (F) show the contour and 3D surface plots for Y_3 of the ibuprofen tablets. A perfect interaction between the factors is characterized by formation of elliptical contours, where the maximum predicted value is identified by the surface confined in the smallest ellipse in the contour diagram (Baril *et al.*, 2010). The ellipse on the contour plot indicates that the interaction effect of the factors CF and DisC on friability is significant (Figure 3.9 (E)). At higher CFs, lower friabilities can be obtained at above medium values of DisCs of the design. The curvature of a contour and the response surface, and the ANOVA results in Table 3.11 ($p = 0.0061$) indicate that the interaction effect of the two factors is significant.

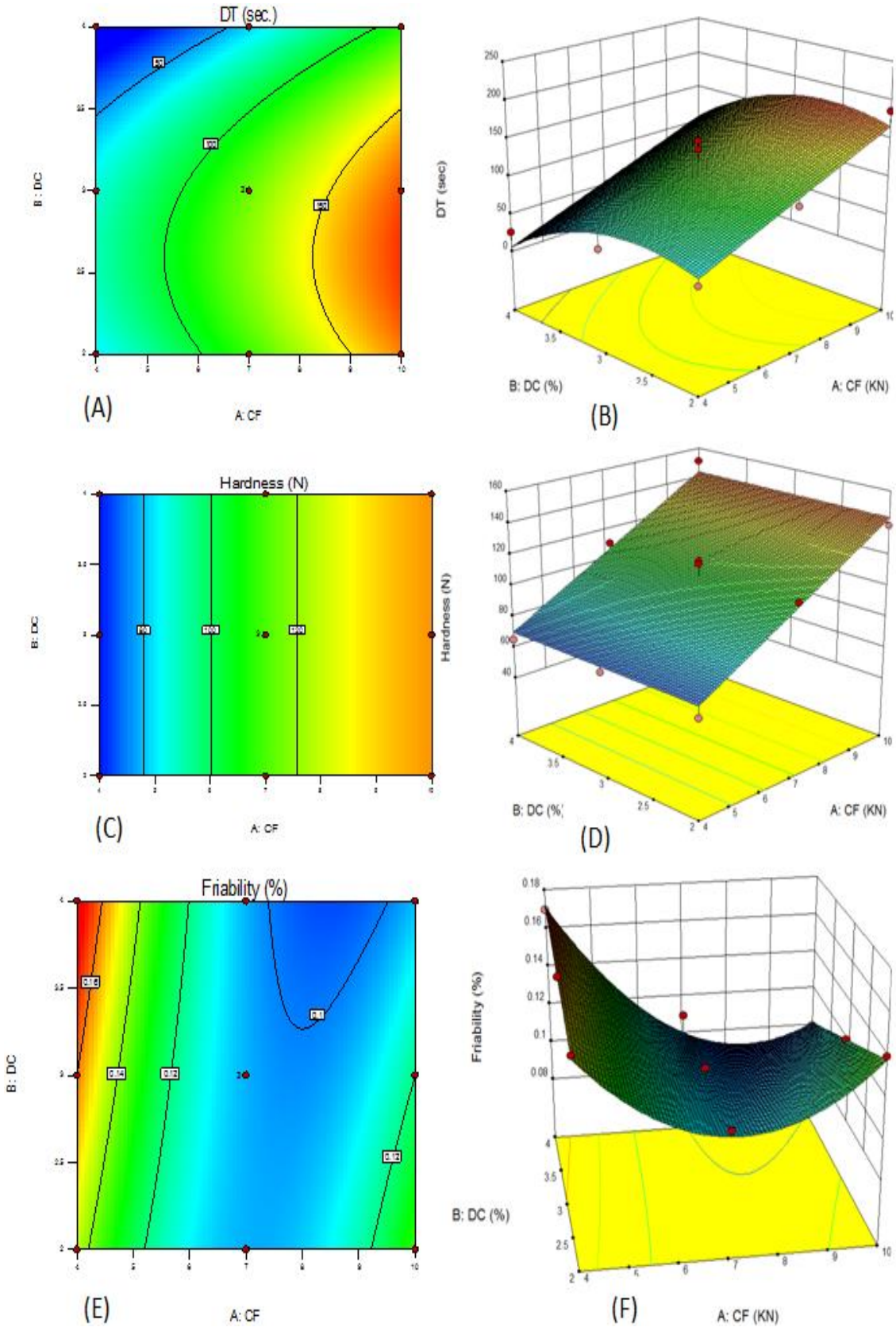

Figure 3.9: Contour and 3D surface plots for DT ((A) and (B)), hardness ((C) and (D) and friability ((E) and (F)), respectively, for the ibuprofen tablets.

3.6.4 Simultaneous Optimization of Responses

In selecting a design optimality criterion for multi-response experiments, all the responses are considered simultaneously. The optimization module of Design Expert® software searches for a combination of factor levels that simultaneously satisfy the criteria placed on each of the responses and factors.

3.6.4.1 Numerical Optimization

One approach for optimizing multiple responses in DOE is the Derringer-Suich method via a desirability function. A geometric mean of desirabilities calculated using desirability functions is used to transform several response variables into a univariate variable (Tsai *et al.*, 2010). Desirability is an objective function that ranges from zero outside of the limits to one at the goal. It reflects the desirable ranges for each response. For several responses and factors, all goals get combined into one desirability function. The value is completely dependent on how closely the lower and upper limits are set relative to the actual optimum. The goal of optimization is to find a good set of conditions that meet all the goals. An optimal formulation product is represented by a maximum desirability value.

Numerical optimization uses the models to search the factor space for the best trade-offs to achieve multiple goals. A numerical optimization technique based on the desirability approach was employed to develop a new formulation with the desired responses. Desirable criteria and goals for the factors and responses respectively were set. Table 3.13 shows the predicted optimum values and the corresponding levels of parameters according to the set goals. Table 3.13 also shows 0.9564 is the overall desirability obtained for the optimum formulation of ibuprofen tablets. Figure 3.10 shows the 3D graph of the overall desirability function.

3.6.4.2 Graphical Optimization

Graphical optimization uses the models to show the volume where acceptable response outcomes can be found. A contour plot is used to characterize the response surface graphically and determine the optimal parameter-setting. When multiple responses are considered, the optimal parameter-setting is obtained by observing overlay contour plots (Tsai *et al.*, 2010). Same goals are set as in the numerical method (Table 3.13). Figure 3.11 shows the overlay plot and confidence intervals superimposed on operating window. As can be observed from

Figure 3.11 (A), overlay plot predicts the optimum same as in the numerical method (Table 3.13).

Table 3.13: Reports of constraints of factors and responses, and solutions of responses of ibuprofen tablets by numerical optimization.

Name	Goal	Lower limit	Upper limit	Importance
A:CF	is in range	4	10	3
B:DC	is in range	2	4	3
DT	minimize	25.3	90	5
Hardness	is in range	50	100	3
Friability	minimize	0.09	0.5	3

Solutions

Number	CF (KN)	DisC (%)	Y_1 (sec)	Y_2 (N)	Y_3 (%)	Desirability	
1	5.151	4.000	25.300	84.261	0.136	0.956	Selected
2	4.000	3.211	63.395	70.159	0.162	0.533	
3	4.000	2.000	64.183	70.159	0.148	0.532	

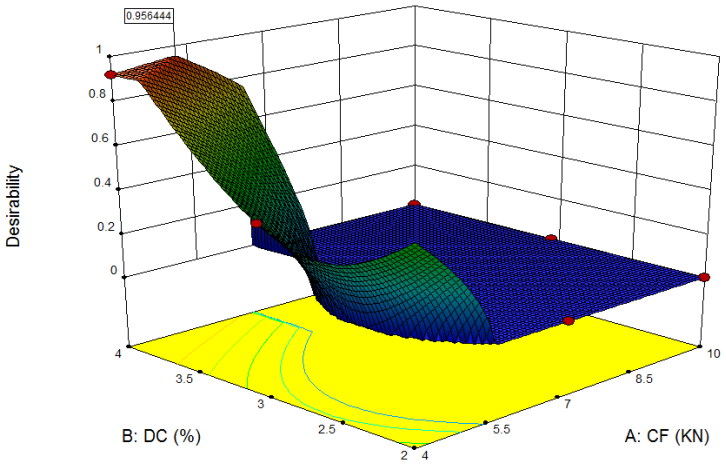

Figure 3.10: 3D graph of desirability function for directly compressed formulation of ibuprofen tablets

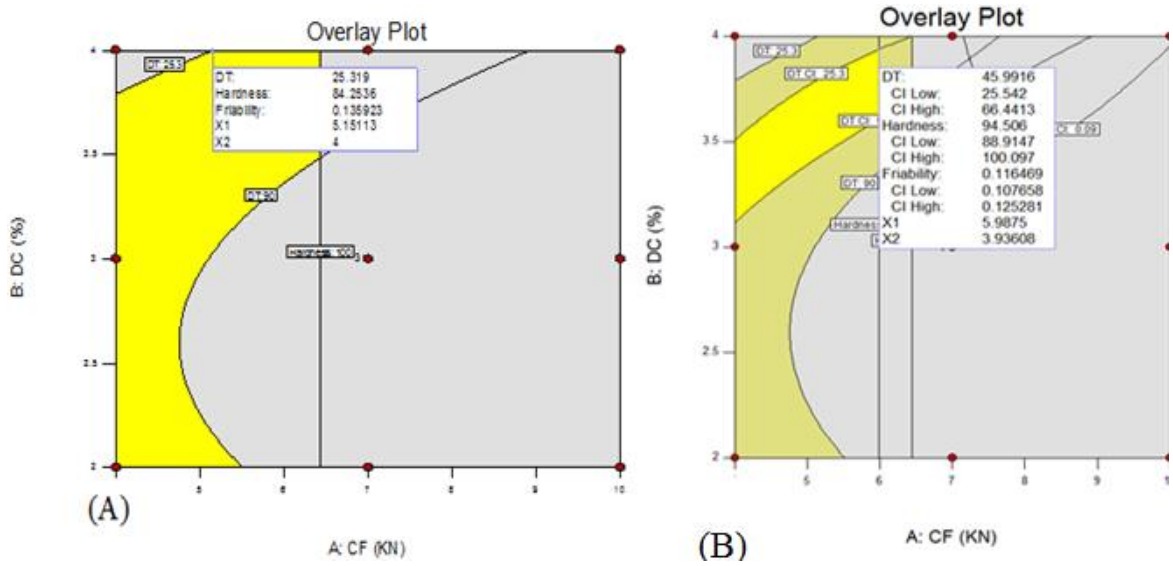

Figure 3.11: Overlying plot (A) and confidence intervals superimposed on operating window (B) of responses as a function of CF and DisC for the directly compressed formulation of ibuprofen tablets identifying optimum level of factors and their responses.

3.6.4.3 Confirmation Test

The results obtained after optimization were verified by conducting the experiments under the optimized conditions of all the factors (Ramakrishna and Susmita, 2012). The confirmation runs were carried out in three batches. If the average observation from the confirmation experiment is within the confirmation node's prediction interval then the model is confirmed. As can be seen from Table 3.14, the values of the data mean column are between the 95% PI low and 95% PI high columns values for each response. This confirms that the experimental values and the predicted values are in good agreement.

Table 3.14: Confirmation report on the optimum formulation variables of ibuprofen tablets ($p < 0.05$, $n = 3$), (SE Pred: Standard error of prediction).

Factor (Name)	Level	Low level	High level
A (CF)	5.13	4.00	10.00
B (DisC)	4.00	2.00	4.00

Response	Pred mean	Pred median	SD	n	SE Pred	95% PI low	Data mean	95% PI high
DT	25.0	25.0	19.84	3	16.96	-15.11	36.10	65.11
Hardness	85.9	85.9	6.92	3	4.88	74.66	76.83	97.17
Friability	0.14	0.14	0.01	3	6.0×10^{-3}	0.13	0.14	0.15

3.7 Evaluation of Tablet Physical Characteristics

3.7.1 Disintegration Time

Tablet disintegration can be regarded as a surrogate for release of the API from the tablet (Augsberger and Hoag, 2008). In this study, the DT ranged from 25.3 - 186.7 sec. The short DT value may guarantee for the dissolution of the ibuprofen tablets within reasonable time.

3.7.2 Hardness

Tablets require a certain amount of strength and resistance to friability, to withstand mechanical shocks of handling in manufacture, packaging, and shipping. Adequate tablet hardness and resistance to powdering and friability are necessary requisites for consumer acceptance. In this study, hardness well above 60 N is attained at lower CFs of about 4 KN.

3.7.3 Friability

The high friability of tablets causes lack of elegance and consumer acceptance. For tablets weighing up to 0.65 g each, a sample of 20 tablets are tested. A maximum loss of 1% is acceptable for most products (BP, 2009). In this study, the friability of the tablets ranged from 0.09 - 0.17% (Table 3.8).

3.7.4 Thickness

At a constant compressive load, tablet thickness varies with changes in die fill, particle size distribution and packing of the particle mix being compressed, and tablet weight, while with a constant die fill, thickness varies with variations in compression forces. As can be seen from Table 3.15, tablet thickness falls within 5% variation of the average.

3.7.5 Weight Variation

With a tablet designed to contain a specific amount of drug in a specific amount of tablet formula, the weight of the tablet being made is measured to ensure that the tablet contains the proper amount of drug. Table 3.15 shows the optimum values of the responses Y_1, Y_2 and Y_3, and weights and thicknesses of the tablets of the three batches from the confirmation runs. No tablet was found to deviate from the average weight by more than 5% (BP, 2009).

Table 3.15: Characteristics of the optimum confirmation formulations of the three batches of the direct compression formulation of ibuprofen tablets.

Batches	Weight (mg)	Thickness (mm)	Y_1	Y_2	Y_3
B1	383.0 (1.2)	4.665 (0.02)	35 (1)	75.9 (2)	0.14
B2	383.2 (1.2)	4.648 (0.01)	36 (1)	76.7 (2.2)	0.13
B3	383.0 (1.2)	4.65 (0.00)	37.3 (0.58)	77.9 (3.1)	0.15
Mean	383(0.12)	4.6543 (0.01)	36.1 (1.2)	76.8 (1.0)	0.140.01)

3.8 Calibration Curve

Figure 3.12 shows the standard calibration curve of ibuprofen. The absorbance of the solution as a function of its concentration was plotted and a calibration curve with a linear regression equation of Y = 0.04081X + 0.01623 (where Y is the absorbance and X is the concentration in µg/ml) and a correlation coefficient of 0.9986 was obtained (Figure 3.12).

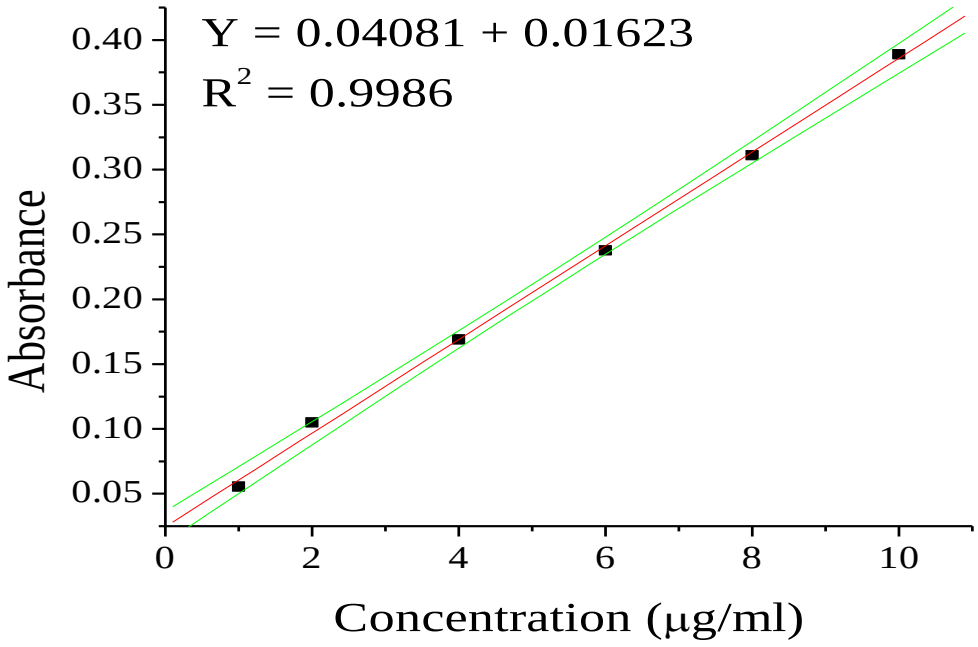

Figure 3.12: Standard calibration curve of ibuprofen in phosphate buffer (pH 7.2) at 221 nm with 95% confidence bands for the mean; (R^2 = 0.9986).

3.9 Dissolution Profile

The dissolution profiles of the ibuprofen tablets of the optimum formulation prepared with 4% ESC as disintegrant compressed at 5.1 KN is shown in Figure 3.13.

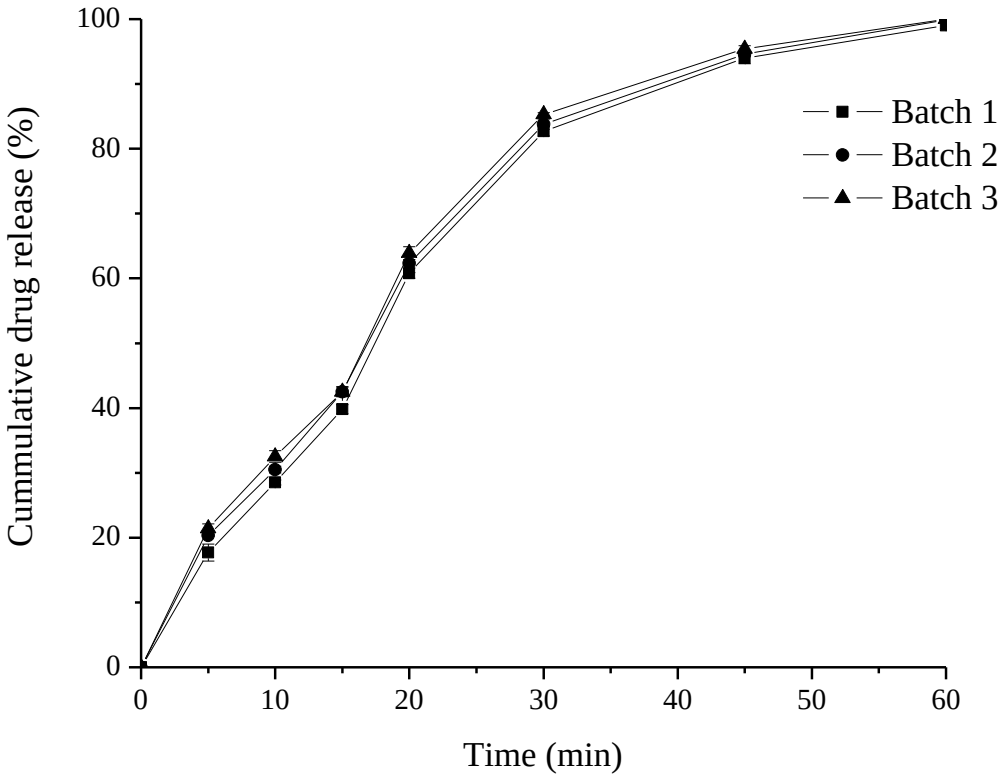

Figure 3.13: Dissolution profiles of 3 batches of ibuprofen tablets.

As can be observed from Figure 3.13, about 84% drug release was achieved within 30 min. This is in contrary to the more than 90% of release found elsewhere (Bushra *et al.*, 2008). This may be because of the hydrophobic glidant, colloidal silicon dioxide, used in this study.

The USP prescribes that 'not less than 80% of the labeled amount of ibuprofen is dissolved in 60 min' as the tolerance limit (USP-30/NF-25, 2007). Bushra *et al*. (2008) formulated ibuprofen tablets in a direct compression formulation employing MCC (Avicel PH 101) as filler-binder, crospovidone as disintegrant and magnesium stearate as lubricant. In most of their formulations, more than 90% of release was attained within 30 min.

4 CONCLUSION

Enset starch citrate (ESC) of desirable physicochemical characteristics was prepared from the modification of starch with citric acid under chemical reaction.

ESC showed better swelling power and hydration capacity than NES to be used as an effective disintegrant. The settling volume of the SCs is significantly higher in DW than in acidic medium. The SCs prepared were more hygroscopic than the native starch. DSC drug-excipient compatibility study demonstrated that there is no interaction between ibuprofen and ESC. Comparison of scanning electron micrograph of the NES and ESC shows there is difference in their granule morphology.

Directly compressed formulation of ibuprofen tablets were successfully formulated using MCC as directly compressible filler-binder, ESC as disintegrant, and colloidal silicon dioxide as glidant. Comparison of ESC and SSG as disintegrants in ibuprofen tablets showed that the tablets with ESC yielded harder tablets than those with SSG, but less effective in disintegration than SSG.

Simultaneous optimization of DT, hardness and friability provided values of 25 sec, 84.3 N and 0.14%, respectively at CF and ESC concentration of 5.2 KN and 4%, respectively, at the optimum point. Dissolution study of the optimized formulation also showed that it fulfilled USP requirements. The optimized formulation was validated to demonstrate that the technique was successful in confirming the predictability and validity of the model.

Therefore, it can be concluded that the ESC can be used as an alternative disintegrant in tablet formulations of practically insoluble drugs.

5 SUGGESTIONS FOR FURTHER WORK

The results of this study suggest further investigations on:

- Rate and extent of water absorption of ESC;
- Evaluation of accelerated and long term stability studies on the optimized formulation of ibuprofen tablets containing ESC as disintegrant;
- ESC as filler in solid dispersion tablets.

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