FORMULATION DEVELOPMENT AND EVALUATION OF HYDROXYUREA DRY SYRUP IN SICKLE CELL DISEASE MANAGEMENT IN PEDIATRICS

Juma Ayubu Mohamedi, (Bpharm)

A Dissertation Submitted in Fulfilment of the Requirements for the Degree of Masters of Pharmacy in Industrial Pharmacy of Muhimbili University of Health and Allied Sciences

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Department of Pharmaceutics and pharmacy practices

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by Muhimbili University of Health and Allied Sciences a dissertation entitled***; Formulation development and evaluation of hydroxyurea dry syrup in sickle cell disease management in pediatrics*** in fulfilment of the requirements for the degree of Masters of Pharmacy in industrial Pharmacy of Muhimbili University of Health and Allied Sciences.

……..…….

Dr. Betty Maganda (Supervisor)

Prof.Eliangiringa Kaale (Co-Supervisor)

Prof.Wei Wang (Co-Supervisor)

DECLARATION AND COPYRIGHT®

I, **Juma Ayubu Mohamedi**, hereby declare that this dissertation is my original work and it has not been presented nor will it be presented to any other University for similar or any other degree award.

Signature: …… Date…….

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I am so grateful to almighty God, for His grace I have completed this work timely.

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DEDICATION

This dissertation is dedicated to my parents Mr. &Mrs. Ayubu Soko and my lovely fiancée Zaria Shuma. Thank you for your love, prayers, tireless support and understanding.

ABSTRACT

Background. Sickle cell disease (SCD) is a genetic disease which affects global population with annual birth of 300,000 SCD infants whom 75% are born in Africa. Tanzania has the highest prevalence (13%) of SCD in the world. Pediatric population is the most affected age group. Currently, hydroxyurea (HU) is the drug of choice for management of SCD but available dosage form exists as capsule with strength of 500mg which fail to match with pediatric dose of 20mg/kg. Current practice of compounding is prone to dose errors and contamination. Also, shortage of compounding laboratories in health facilities in the developing countries the major issue.

Aim. The aim of this study was to develop and evaluate the HU dry syrup formulation for the management of SCD in pediatrics.

Methodology. Preformulation and formulation phases involved use of high-performance liquid chromatography (HPLC), Fourier transform infrared spectroscopy (FITR) rotational rheometers, USP type II dissolution apparatus, digital pH meters. Evaluation of dry syrup involved measurement of angle of repose, compressibility index, Hausner's ratio, assay, dissolution and moisture content. The reconstituted syrup was then subjected to in use stability testing as part of evaluation. In that case the reconstituted syrup was evaluated for duration of 14 days for its pH, viscosity and assay.

Results. All the formulations showed promising physicochemical features which were in agreement with pharmacopeia range, excellent in flowability, assay, dissolution, pseudoplastic flow property and good pH.

Conclusion. The HU dry syrup formulation was successfully developed, optimized and evaluated. It has a potential to address the dose challenges in pediatric population. Further studies on important issues such as stability studies and formulation up-scaling are recommended.

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LIST OF ABBREVIATIONS OF COMMONLY USED ACRONYMS

1.	ADRs	Adverse drug reactions
2.	API	Active pharmaceutical ingredient
3.	BP	British Pharmacopeia
4.	DDS	Drug delivery system
5.	DT	Dissolution testing
6.	FPP	Finished pharmaceutical product
7.	FTIR	Fourier transform infra-red spectrophotometer
8.	Hb	Hemoglobin
9.	HbA	Adult hemoglobin
10.	HbF	Fetal hemoglobin
11.	HPLC	High performance liquid chromatography
12.	HPTLC	High performance thin layer Chromatography
13.	HU	Hydroxyurea
14.	LOD	Loss on drying
15.	MC	Moisture content
16.	NMRA	National medicines regulatory authority
17.	PYO	Personal years observational
18.	TLC	Thin layer chromatography
19.	USP	United States Pharmacopeia

DEFINITION OF KEY TERMS

Adult hemoglobin-Is the type of human Hb which is made up of two α chains and two β chains, this type of Hb predominates after six months of life to death. This type of Hb can be affected by sickle cell disease.

Dry syrup-This is the type of pharmaceutical dosage form existing as a powder blend or granules which need to be reconstituted at the time of administration. Many aqueous unstable drugs are manufactured in this form to prolong stability.

Fetal hemoglobin-Is the type of human Hb made up of two α chains and two δ chains. This Hb predominates in the first days of life and get replaced with adult Hb after six months of life. This type of Hb is not affected by Sickle cell disease.

Hydroxyurea -This is antineoplastic drug which belong to antimetabolite group used for management of leukemia. The drug is very old as it was used in leukemia but in 1990s got approval to be used in management of sickle cell disease.

Pediatric population -Is the group of individuals whose age is below 18 years of age, this group is further subdivided into neonates who are 0-1month, infants who are 0-1-year, young children who are 2-6 years, children who are 6-11 years and adolescents who are 11-18 years. Nevertheless, in physiology pediatric population is defined as individual who are below 12 years of age.

Sickle cell disease -This is a genetic disease caused by mutation in a single nucleotide which code for amino acid glutamate replaced by valine in the six position of the β- globin chain of hemoglobin. This genetic change will cause the shape of red blood cell to be deformed. The deformed red blood cell will occlude blood vessels in areas of low oxygen tension and cause severe pain and sometimes end organ damage a condition known as crisis.

CHAPTER ONE

1. INTRODUCTION

1.1. Back ground

Sickle cell disease (SCD) is a genetic disease which affects global population but it has a greater magnitude in Africa. The annual birth of infants with SCD is 300,000 infants worldwide of which 75% of them are born in Africa (1). Among the African countries Tanzania has the highest prevalence of SCD particularly in the north western regions including Kagera, Mwanza, and Mara (2).Although the disease has higher prevalence in Africa the current trend of voluntary migration places the world in danger for higher cases. The impact of voluntary migration was evidenced by increasing number of migrants who carried a gene for SCD from 1.6 million in 1960 to 3.6million worldwide in 2000 (3). This new trend of SCD, forced World health organization (WHO) to urge every country to have its own strategy of reducing number of SCD individual. The prevention strategies includes genetic counseling among couples and newborn screening for all children (4). The morbidity and mortality pattern differ a lot between the developed country and developing countries. Existence of highly improved healthcare system in developed countries allow 95% of children with SCD to reach the adulthood (3). On the other hand, in developing countries due to poor healthcare systems, death rate amongst children with SCD is high and most of them die during first three years of their life (1). Therefore; the combating efforts should be directed to this age group.

SCD is caused by mutation in the β-chain of hemoglobin (Hb) where correct amino acid glutamate is replaced with valine. This replacement causes the polymerization of Hb hence, deforming the shape of red blood cells (RBCs) (5). The deformed RBCs assume sickle shape and will have many abnormal features such as membrane stability problems, easily dehydrated and easily adhering to vascular endothelium (3).Therefore SCD has two major manifestations which are vaso-occlusive crisis and aplastic crisis or hemolytic anemia (3). Occlusion of blood vessels by deformed RBC in areas of low oxygen tension cause the release of mediators of inflammation which results into very severe musculoskeletal pain. These manifestations range from severe pain in the bones ,acute chest syndrome ,priapism and finally ischemic end organ damage either to the lung, kidney or brain (6). Among the occlusion complications, acute chest syndrome is the most lethal

as it causes death to many SCD patients. The recurrence of these manifestations is mainly contributed by infections. It is important to note that the complications of SCD will not be apparent in the first six months of life because at this point fetal hemoglobin (HbF) predominates but later on adult hemoglobin (HbA) will predominate by 97% and a child will face the sequelae (7). Pediatric population are highly susceptible to infections either due to underdeveloped immunity or because of malnutrition (8).The mortality and morbidity due to SCD are extremely pronounced to pediatric population and many children die within the first three years of life (1).

Management of SCD is directed into either prophylaxis or symptomatic therapy. Many SCD patient take prophylactic dose of penicillin for prevention of infections which are aggravating factor for occlusive events (8). Symptomatic treatment for severe pain ranges from non-steroidal anti-inflammatory drugs (NSAIDS) to opioids (4). However, this symptomatic management is found to be insufficient and prolongs use of NSAIDS that increases the chances of gastrointestinal tract corrosion.

Chronic hemolytic anemia and occlusive events necessitate several hospitalizations and blood transfusions which may result into iron overload (6). These challenges have been solved by introduction of hydroxyl urea (HU). Standard treatment guidelines of Tanzania of 2017 recommend a dose of 20mg/kg of HU for management of SCD (2) . Worldwide, HU is mainly found in capsule of 500mg which is not very appropriate formulation to pediatric population (4). Capsules are difficult to swallow and the available strengths are too big to match with body weights of children.

HU is antineoplastic agent which was firstly synthesized by two Germany scientists namely Dressler and Stein in 1869 (9). The drug was approved by Food and Drug administration (FDA) for management of tumors particularly chronic myelogenous leukemia in 1967.Then in 1998 FDA approved additional indication which was SCD in adult. Following safety assessment of HU in infants the drug was then approved by FDA in 2002 to be used in the management of SCD in pediatric population (9) . The mechanism of action of HU in SCD is not very clear but the drug inhibits ribonucleotide reductase and induce the synthesis of HbF (9). Since HbF has only α-chain and δ-chain the sequalae of SCD are evaded because SCD affect β-chain. Additional advantages of HU include increase of mean corpuscular volume (MCV), reduction of reticulocytes counts,

intracellular dehydration of RBC, adherence tendency of RBC to vascular endothelium and finally produce nitric oxide which is a potent vasodilator (5). Thus, combination of those mechanism will increase Hb concentration and HbF. HU has proven to reduce the rate of both blood transfusion and hospitalization hence improve the quality of life of SCD patients (10).

Dry syrup formulation refers to the dosage form which need to be reconstituted at the time of administration and they are intended to be taken orally (11). Chemical and physical instability of API is the driving factor for selection of such dosage form. In a situation where the API cannot be able to resist hydrolysis for a period of 7-14 days then conventional syrup cannot be formulated (12). Therefore, Dry syrup either in granules or powder blend are formulated to maintain chemical stability by reducing the time in which API is exposed to aqueous environment. The technical details of dry syrup are shown in section 1.8.

1.2. Problem statement

Currently, SCD is the most lethal disease among non-communicable disease (NCD) as far as pediatric population is concerned (1). In spite of WHO strategies to reduce mortality to under 5 children by combating the communicable diseases, their rate is still high due to SCD (13). The global mortality rate due to SCD among pediatrics is 0.64% years person observation (PYO) while in Africa mortality rate is 7.3% PYO (14).Tanzania has the highest annual birth of SCD infants in the world reaching 11000 birth per year this is due to high prevalence of Hbs gene among Tanzanian which is 13% (15). In developing countries such as Tanzania 50-90% of SCD infants will die during child hood if strong interventions in improving health care delivery will not be properly implemented (14).

HU is currently the drug of choice for management of SCD but it is available only in capsule form to a strength of 500mg (4).The challenge is that the strength of 500 mg is too big to match with pediatric dose of 20mg/kg (16). To overcome this challenge the compounding or extemporaneous preparation is done. However, the main issue with compounding practice is the coverage of entire population due to fact that very few health facilities have compounding unit in developing countries. Apart from compounding the other practices include capsule opening and dissolving the content in water or hot water,

approximating the dose to nearest 250mg or 500mg capsule. The later practice had a lot of drawbacks such as dose inaccuracy due to wastage of some powder during opening and drug degradation when using hot water (17). Dose inaccuracy can compromise the treatment outcome and or increase the risk of adverse drug reactions (ADRs). Also, capsules are neither sweetened nor flavored. Therefore, bitterness of medication may have psychological impact to the children and affect adherence to treatment.

To improve the treatment outcomes and so the quality of life of SCD children there is a need to develop a liquid formulation of HU which allow dosage individualization according to child's weight. Dry syrup formulation is preferred over conventional syrup due to ease of storage, transportation and enhanced stability as the API is exposed to aqueous environment only at the time of administration.

1.3. Conceptual framework

Lack of pediatric friendly formulation of HU in the market is the major challenge in the management of SCD in Children. The current available formulation which is a capsule has many drawbacks as far as pediatric population is concerned. Successfully developed dry syrup is expected to have number of positive outcomes in the management of SCD. The primary independent variables for dry syrup development are pre-formulation studies and formulation development which will result into number of formulations of dry syrup of HU. Secondary independent variable will be evaluation parameters which will result into formulation of dry syrup of HU of desired features .Tertiary independent variables are easy administration, dosage individualization, flexible dosage , easy transport and easy storage which will result into dependent variables which are improved adherence to medication due to good taste ,reduced side effect and ADR due to dose individualization, improve treatment outcome, reduce hospitalization hence improve quality of life of SCD children. The relationship between these variables are shown in Figure 1.

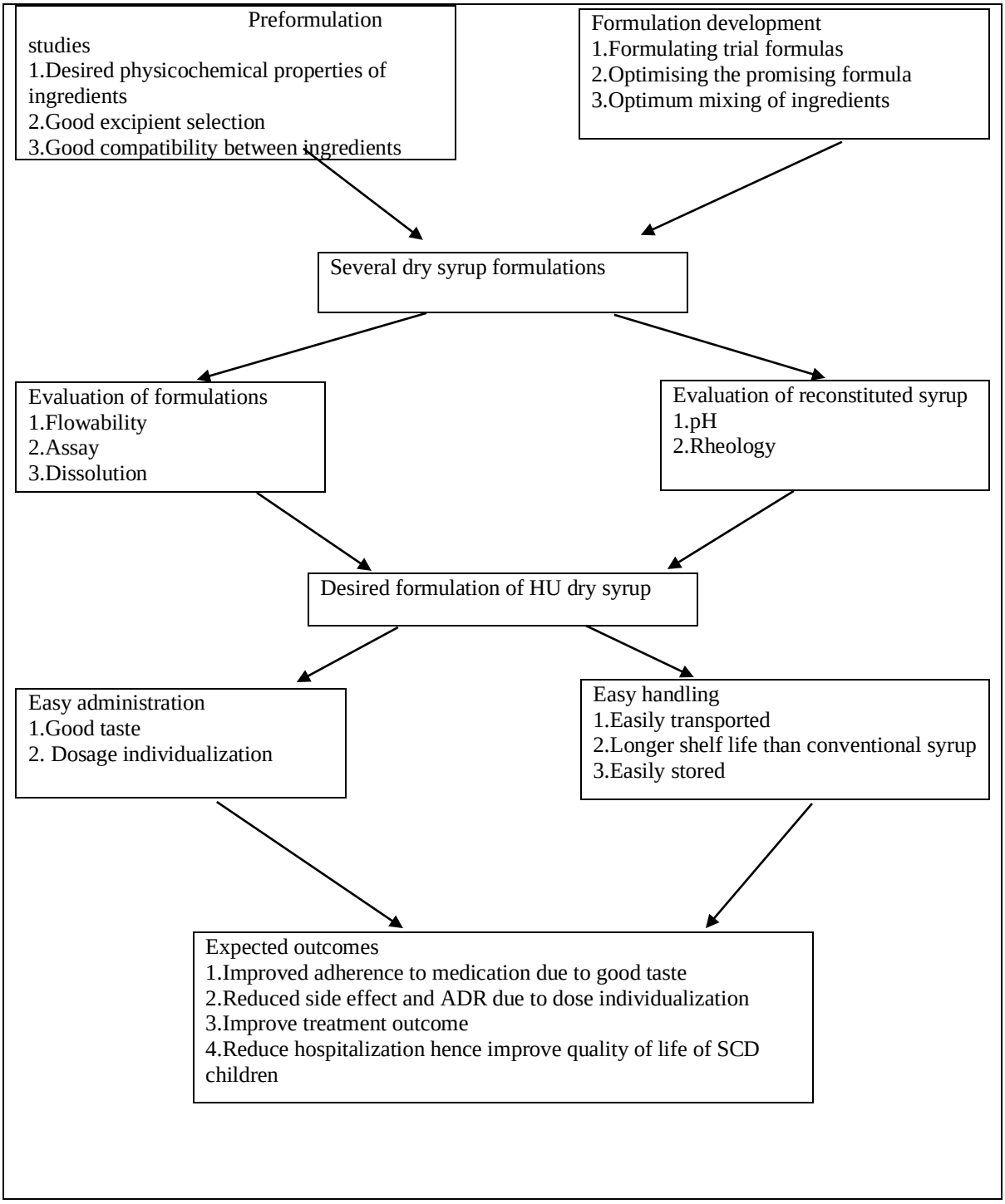


Figure 1:Interaction between dependent variable and independent variables leading to successfully formulation.

1.4. Rationale of the study

The findings from this study has filled the gap and added knowledge to the science because the unknown information about HU and possibility of formulating it into dry syrup is now clear. These findings will be used in up-scaling from laboratory scale production to

industrial scale production. Furthermore, this study has created awareness on SCD in pediatric population as well as enthusiasm into the molecule of HU and its finished pharmaceutical product (FPP) therefore, has invited many studies to be done until the permanent solution in management of SCD in pediatric population is found.

1.5. Research questions

The following questions were the driving factor for doing this research of developing HU dry syrup formulation for pediatric population.

i. Will HU be stable in aqueous environment and for how long?
ii. Which excipients will not react with HU in the formulation?
iii. What will be the most optimal formulation of HU dry syrup?
iv. How will dry syrup and its reconstituted syrup behave physically and chemically?

1.6. Broad objective

To develop and evaluate the HU dry syrup formulation for management of sickle cell disease in pediatric population.

1.7. Specific objectives

i. To determine the hydrolysis kinetics of aqueous hydroxyurea.
ii. To assess the chemical compatibility between HU and potential ingredients.
iii. To develop the optimum formulation of HU dry syrup.
iv. To evaluate the physicochemical properties of dry syrup and its reconstituted syrup.

1.8. Literature review

HU is an antineoplastic agent that belong to antimetabolite group which act by inhibiting ribonucleotide reductase an enzyme which converts ribonucleotides to deoxyribose nucleic acid (DNA) (7). HU depletes DNA in rapid growing cells such as tumor cells to be precise it inhibits S-phase of cell cycle (10). By inhibiting S-phase HU got many clinical applications in management of tumors of head and neck, polycythemia conditions and chronic myelogenous leukemia. HU was also used in management of HIV/AIDS though, it was immediately replaced by combination regimen of antiretroviral drugs (18). Later on, the drug was found to induce synthesis of HbF and got value in management of SCD. The

clinical benefits of HU in management of SCD were reduced episode of vascular occlusive events and blood transfusion, reduced rate of hospitalization and prevention of end organ damage all of which resulted into improvement in quality of life of SCD patients (19). The side effects of HU are dose dependent and common ones includes neutropenia ,reticulocytopenia, and thrombocytopenia (18). Various pharmacokinetics studies of HU have shown that has oral bioavailability ranges from 75 to 79%. It is rapidly absorbed from the gastrointestinal tract by passive diffusion and peak plasma concentration is achieved within 3-4 hours. It has volume of distribution equal to body water. Its elimination is both renal and non-renal mechanism whereas renal mechanism predominates. The drug has renal clearance which is 75% of glomerular filtration rate (10).

Currently, the available formulation of HU is only the solid dosage form, which is capsule of 200mg, 250mg, 300mg ,500mg, still the most available strength on the market is of 500mg. (18). The common excipients which are incorporated in these capsules includes gelatin, citrate, lactose, erythrose, magnesium stearate iron oxide, sodium lauryl ether sulphate, sodium monohydrogen phosphate, titanium and aerosil. So far, there is no liquid formulation of HU for pediatric population in the market despite a great demand for this age group (19).

The known physicochemical properties of HU molecule includes the following:-white crystalline powder, tasteless, highly water soluble but insoluble in alcohol, very hygroscopic and decomposes in the presence of moisture (18). The aqueous instability and hygroscopicity are probably the major factors which force many companies to produce solid dosage form. The chemical structure of HU which is hydroxycarbamide is as shown in Figure 2.

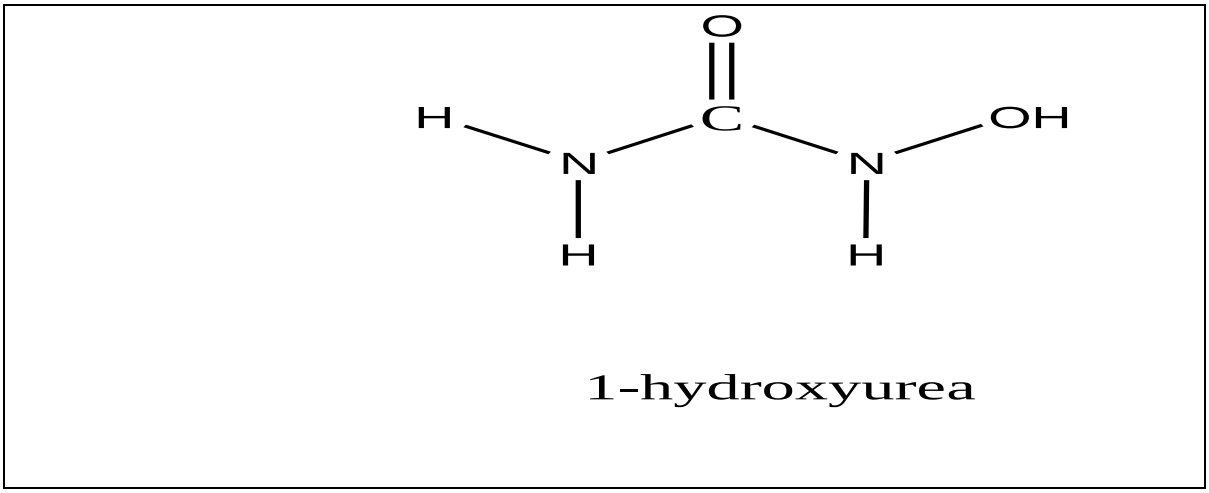


Figure 2: Chemical structure of hydroxyurea molecule.

The general order of hydrolysis reaction starts from esters which are most susceptible followed by amide and then other bond (20). Nevertheless, information about rate and extent of hydrolysis of HU in aqueous solution is yet to be reported (19). Establishing the kinetic hydrolysis of HU in aqueous solution will predict the possibility of developing either a syrup or dry syrup basing on the order of kinetic and hydrolysis constant "K" to be determined. Conversely, preliminary physicochemical data are suggesting a possibility of dry syrup rather than syrup.

There are two analytical techniques for quantification of hydroxyurea which are chromatographic and spectrophotometric technique with ultraviolet detectors (22,23).High performance liquid chromatography (HPLC) run under reverse phase that allows HU a polar molecule to be eluted in the column and basing on the consideration of peak areas the quantification become possible (23). Qualitative techniques involve use of infrared spectrophotometer for identification purposes. Mid infrared in the range of wave length $4000cm^{-1}$ to $400cm^{-1}$ can give the vibrational frequencies corresponding to the functional group present in different ingredients and produce spectra (24). Comparisons of those spectra with the library help in identification and compatibility studies.

Dry syrup refers to powder blends or granules which are reconstituted at the time of consumption. A dry syrup should be able to maintain chemical and physical stability until end of regimen particularly 14 days (25).This type of dosage form is becoming indispensable option when the drug cannot be exposed to aqueous environment for a long time due to stability issues. For aqueous unstable drug like HU, formulating a dry syrup

will have many advantages such as, maintaining physical and chemical stability for desired period of time. Also, it reduces the weight and facilitates transportation of FPP. Inclusion of sweeteners and flavors in dry syrup increases acceptability to pediatric population. The most important advantage is dosage individualization due to the fact that dry syrup upon reconstitution become flexible to any dose depending on the child's body weight. It should be noted that HU is antineoplastic agent with carcinogenic potential so individualized dose will improve treatment outcomes, reduce ADRs such as the risk of developing malignancy later on (11). Dry syrups are generally categorized into two categories namely powder blend and powder granules depending on both components and method of production. Powder blend are simply produced by dry mixing or blending of API and excipients in the suitable mixer with the optimum mixing time. Powder blend is becoming a good choice when the drug is highly hygroscopic and aqueous unstable because FPP will have less moisture content (11).The disadvantage of powder blend is that the materials can easily segregate. On the other hand, powder granules are produced by two steps start with mixing of API and excipients followed by massing of blend with granulating solution or liquid either ethanol, isopropyl alcohol or water. The advantages of powder granules include improved flowability and decreased chance of demixing or segregation (26). Necessary excipients for dry syrup includes suspending agents, colouring agents, sweetener, buffers, anti-caking agent, flavouring agents and preservatives (27). It's very important that the excipients are compatible and protect the API from rapid degradation in the formulation. Xanthan gum is the ideal suspending agent for most of dry syrup because of its ability to maintain viscosity in a wide range of temperature and pH (28). Colouring agents are normally included to give elegancy to the formulation. However, there have been many health concerns regarding inclusion of colouring agent especially in pediatric population. Many colouring agents have carcinogenic potential hence avoiding them it's a wise decision (28, 29). D-mannitol is one of the bulky sweeteners which is the best option for preparations for pediatric population as it avoids dental carries and do not rise the blood sugar levels (30,31,32). Nevertheless, because it is non hygroscopic it can protect the hygroscopic API such as HU from absorbing moisture (33). Sodium citrate is a good buffering agent because of its dual effect which avoids pH variation of the formulation and act as antioxidant too (34). Sodium benzoate is the preferred preservative for pediatric preparation because it is relatively safer than parabens. Parabens are associated with

inhibition of normal endocrine function hence are contraindicated in pediatric population (27).

Glidants and anticaking inclusion is mandatory in formulation with hygroscopic product to prevent moisture entrapments which can lead to caking. Aerosil 200 is one of the best anticaking agents because of its double function as it acts as glidant to improve flowability and at the same time acts as desiccating agent to prevent moisture accumulation in the product (35).

The need for dry syrup formulation of HU is supported by number of studies such as study done by Jeremiah et al 2016 That study indicated the need of producing liquid formulation of hydroxyurea so as to improve treatment outcomes of SCD. His findings concluded that the HU solution and capsule were both bioequivalent but due to a number of challenges accompanied with capsule, formulating a liquid formulation such as dry syrup is paramount (10).

Despite the number of studies done on HU suggesting its preparation in liquid formulation to date only solid dosage form is available on the market, particularly capsule (7). Lack of information on hydrolysis kinetics of aqueous solution of HU is probably the major limiting factor hindering possibility of developing into liquid formulation. Also, the drug has been tested for common excipients of capsule dosage form but did not test for other dosage forms. Therefore, there is a need to establish compatibility with excipients of liquid dosage form such as sweetener, flavouring and colouring agents so as to allow possibility of making other dosage forms. Detailed study on flowability of HU-formulation in powdered formulation is yet to be established for dry syrup. The following paragraphs will detail the literature behind every specific objective as follows and outline known and unknown information.

1.8.1The hydrolysis kinetics of aqueous hydroxyurea

For the drug which requires reconstitution the hydrolysis kinetic in aqueous solution is essential for predicting in use stability. The findings of hydrolysis kinetics provide information on product stability upon reconstitution. As far as dry syrup is concerned it must be able to resist hydrolysis upon reconstitution for not less than 14 days (36).The probable hydrolysis reaction of hydroxyurea is the one leading to formation of

hydroxylamine as described by Heeney et al 2008 (19) which resulted in loss of its therapeutic activity as shown in Figure 3.

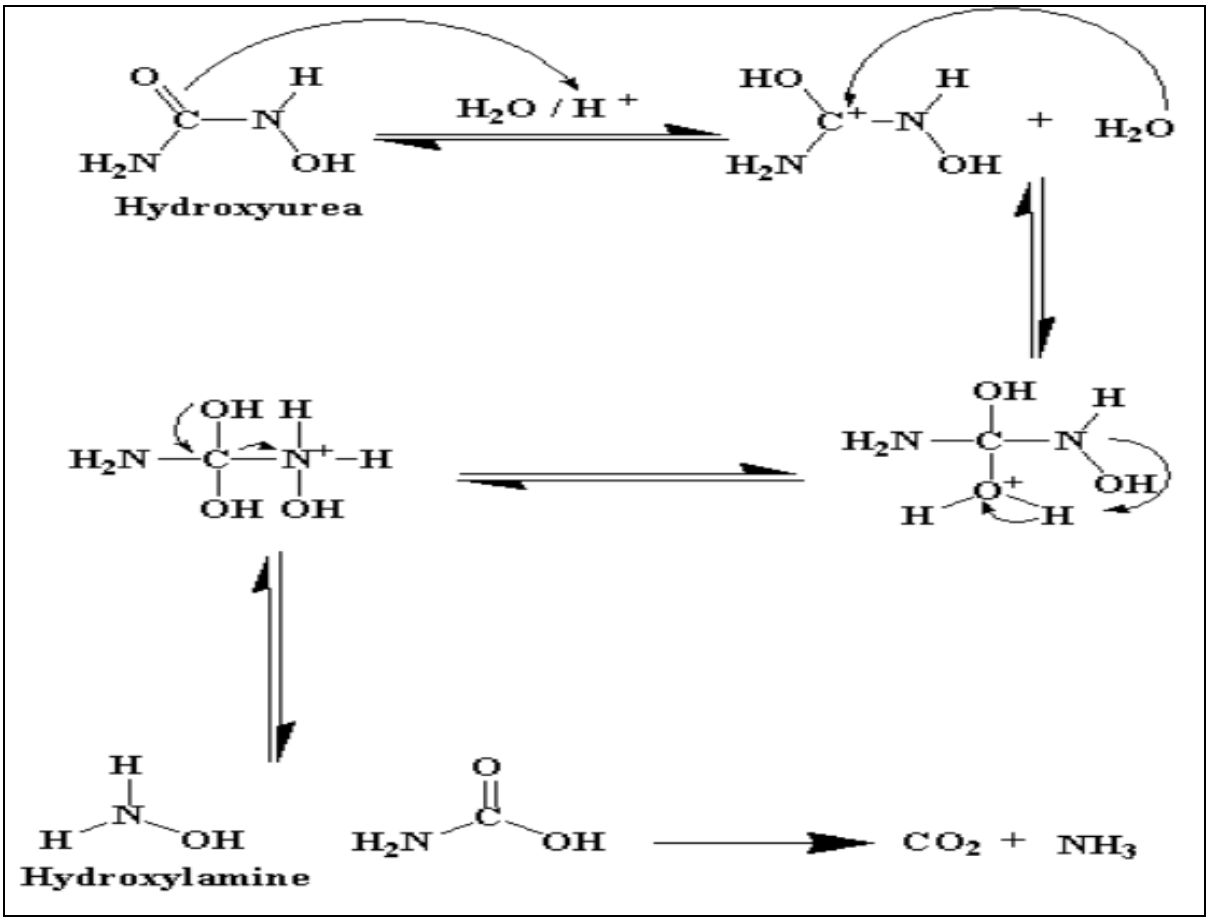


Figure 3: Hydrolysis reaction of hydroxyurea to form hydroxylamine which lost therapeutic activity.

To predict the in-use stability of dry syrup upon reconstitution the kinetic study is always conducted at different conditions of temperature. The commonly used conditions are refrigeration (4°C), room temperature (25°C) and extreme high temperature of 50°C.Normally the kinetic studies are conducted with the help of HPLC as a drug quantification tool (18).The quantified drug concentration are then studied for a given time to predict the nature of kinetic and its kinetic constant.

1.8.2. Chemical compatibility between API and potential ingredients

Chemical compatibility testing is part of preformulation studies which ensure absence of chemical reactions among API and excipients in the formulation. There are different methods for conducting chemical compatibility such as differential scanning colorimetry, thermogravimetric analysis, HPLC, Fourier transform infrared spectrophotometer (FTIR)

and thin layer chromatography (TLC). FTIR scans give the wave number in the range of 4000-400 cm^{-1} for different functional groups (24). From the fact that when a chemical reaction happens there will be a formation of new functional group so the spectra will be different from original spectra. HPLC is used for compatibility studies by investigating formation of new peaks, if there is any chemical reaction in a compound new peak forms which normally have different retention time.

1.8.3. Formulation optimization

Development of optimum formulation involves safety and stability consideration during excipients selection. The excipients should be selected in such a way that the API is maximally protected in the formulation and also, they should be safe to the intended age group. Xanthan gum is **a** suitable thickener for most dry syrups because of its shear thinning property, also the viscosity of this gum is not affected by temperature and pH conditions of the formulation (28). The shear thinning property of this gum allows easy pourability after shaking the formulation. D-Mannitol is the best sweetener if the drug is to be used for chronic diseases. This kind of sweetener is not metabolized by cariogenic bacteria hence dental carries will not be a problem even with prolonged use (33). Furthermore, this sweetener does not raise the blood sugar level. Though sorbitol has almost similar properties as D-Mannitol what makes D-Mannitol more preferable is its non-hygroscopicity (37). Since HU is highly hygroscopic and decomposes in aqueous environment, using D-Mannitol will protect this API from absorbing more moisture. Among the buffers Sodium citrate is preferred because of its triple role, acting as a buffer, lowering the pH and acts as antioxidant. Sodium benzoate is the preservative of choice for pediatric formulation because it is relative safer than parabens. Desiccant inclusion is mandatory for hygroscopic APIs in the powdered formulation to prevent caking. Aerosil 200 by having dual role has now become important desiccant. Aerosil 200 can act both as desiccant and glidant to improve the flowability of the powdered formulation. Inclusion of coloring agents and flavors has raised many health concerns especially proven carcinogenic potential of some colouring agents. From the fact that HU is tasteless drug so the sweetener alone without flavouring agent probably suffices.

Dry syrups are commonly manufactured as powder blends or granules. The granulation technique is either wet granulation or dry granulation. Despite the granules having superior properties such as good flowability, do not segregate easily upon mixing and preventing dusting out effect the API features but technological capability limits its selection. Because HU is hygroscopic and unstable in aqueous environment, though isopropanol could be used but technology limitation forces HU to be formulated as powder blends rather than granules. The powder blends manufacturing involves milling, sieving then followed by mixing in the appropriate mixer or blender with optimum mixing time to prevent demixing. The details of procedures will be given in the methodology section.

1.8.4. Physicochemical properties of HU dry syrup

Flowability is the most important physical property of either powder blend or granules. The flowability influences uniform filling of powder blends in either capsules or bottle container from the hopper. Flowability is determined by parameters such as angle of repose, bulk volume, tapped volume, bulk density, tapped density, compressibility index and Hausner's ratio (26). The detail of each parameter will be given in the methodology section. The other important properties are assay, dissolution, and moisture content. Assay or drug content measures the percentage of API in the formulation. Basing on USP the allowable range is 90% to 110%.Sometimes assay may reflect the dosage uniformity of the formulation (19). Dosage uniformity for different portion of the powder blend is also an indicator that the mixing is optimal and the API is uniformly distributed in the batch. Dissolution or invitro drug release is the measure of how fast the drug delivery system releases the API in the GIT for rapid absorption. USP requires the formulation to release more than 85% of the API in the first 30minutes (25).Moisture content is the measure of the volatile components and water molecules by the API or FPP, for the API the moisture content is intrinsic property of the API, while for the FPP the moisture content is affected by manufacturing processes. The moisture content should be as low as possible to limit the microbial growth in the formulation and reducing rate and extent of hydrolysis.

1.8.5. Physicochemical properties of reconstituted dry syrup

Viscosity, sedimentation volume and pH are important properties which demonstrate the physicochemical stability of the formulation. Stable dry syrup upon reconstitution should be able to maintain its viscosity and pH for at least 14 days without showing significant

change. The change in viscosity and/or pH is an indicator of either depolymerization of the thickener which might be caused by microbial growth or chemical reaction. Sedimentation volume is another indicator of physical stability of the formulation. Sedimentation volume is only determined when dealing with suspension, but if both API and excipients dissolve and form a clear solution then it becomes obsolete.

CHAPTER TWO

2. METHODS

2.1. Study design

The experimental study design was used which involved pre-formulation studies, formulation optimization and evaluation. The experiments were conducted at the chemistry department of the University in Bergen-Norway as well as research and development laboratory of Muhimbili University in the School of Pharmacy because of its reputations in doing those experiments and technical capability.

2.2. Reagents and chemicals

Analytical grade of reagents and chemicals were purchased from sigma- Aldrich life science company in the city of Bergen Norway. The reagents procured were as follows **:**Hydroxyurea powder, Uracil powder, D-Mannitol powder, Sodium citrate powder, Methanol 98% v/v, Acetonitrile, Potassium bromide and Xanthan gum powder manufactured by Sigma life science- China, Sodium benzoate powder was manufactured by Sigma life science -Netherland, Aerosil 200 was manufactured by Evonic industries-Japan.

2.3. Instrumentation

The following instruments and equipment were used during the experiments: HPLC-Agilent technologies version Agilent 1260 infinity II with both UV and diode array detectors from United States of America (USA). HPLC was running under reverse phase in isocratic mode at flow rate of 1ml/min. The UV detectors were set at 214nm and 254nm.FTIR spectrophotometer produced by Thermo Fischer-scientific, version Nicolet IS50R - United Kingdom. The FTIR Linked to attenuated total reflectance (ATR) was set to produce 32 scans per spectra with a resolution of $4cm^{-1}$. Kinexus rotational rheometer produced by Malvern -United Kingdom. The specific disc diameter of 60mm rotated at 100rpm was chosen and allowed to generate shear on the surface of 1mls of reconstituted syrup placed on flat surface. Dissolution testing machine was USP type II paddle apparatus produced by Erweka-USA. The apparatus was set to produce 50rpm as per USP. The pH meter used was digital one from Mettler Toledo -USA.

2.4. Safety concerns and personal protective equipment

It's important to understand that Hydroxyurea is antineoplastic agent so all experiment involving this drug were done in the fume hood. Personal protective equipment were used in accordance with material safety data sheet and these included rubber chemical resistant gloves made by Sigma life science in China, safety goggles' from Mettler Toledo - USA, laboratory coat and NIOSH mask respirators made from Agilent technologies company - USA (38).

2.5. Hydrolysis kinetics of aqueous hydroxyurea

2.5.1. Calibration curve development

Stock solution of 2mg/ml of HU solution was made by dissolving 20mg of HU crystalline powder with sterile distilled water in volumetric flask up to 20ml. Internal standard solution was made by dissolving 12.5mg of Uracil powder with sterile distilled water into 10mls volumetric flask to make 1.25mg/ml of Uracil solution. The stock solution was serially diluted to attain seven standard calibration point with concentration of HU ranging 0.015625 mg/ml to 2mg/ml and internal standard 0.25mg/ml uracil. The detailed procedures for calibration curve development are found in appendix i.

2.5.2. Quantification of HU in aqueous solution

The hydrolysis kinetics of aqueous Hydroxyurea was studied by minimum modification of the validated HPLC -assay method described by Plusce and Yuan (23) .The method was used to quantify three stock solutions of HU of 2mg/ml namely X, Y and Z stored at different condition of temperature 4°C, 22°C and 50°C respectively. This study was conducted for 90 days and finally the plot of concentration against time was investigated to give the nature and constant of the kinetics. The detailed procedures for quantification of HU are found in appendix ii.

2.6. Chemical compatibility studies

2.6.1. Infra-red spectroscopic technique

The Fourier transform Infrared spectrophotometer at the range of 4000-400cm^{-1} under ambient temperature was used to assess the chemical compatibility between API and potential excipients (24). Minor modification of the method described by Venkateswarlu was used to prepare the powder mixture for FTIR spectra production and analysis (37) . The spectrum of hydroxyurea European Pharmacopeia reference standard was produced and compared with the spectra of purchased API to act as identification test and

compatibility test. API was mixed with each and every excipient in 1:10 then spectra were acquired on the 1st day, these powdered mixtures were placed at in the stability chamber at 40 ±2°C and 75% ±5%RH for a period of 4 weeks .On the end of 4th week the spectra were acquired again and compared with spectra of first day.

2.6.2. HPLC technique to support FTIR and increasing acceptance criteria

The API-Excipient mixture in the 1:10 was dissolved in distilled water, filtered and placed in HPLC vials. The HPLC was run at 1ml/min in a reverse phase model, whereby distilled water was used as a mobile phase. The chromatograms produced were kept for comparison. The other half each mixture was placed in the stability chamber at 40 ±2°C and 75% ±5%RH for a period of 4 weeks. After 4 weeks the HPLC was ran again and chromatograms produced were compared with initial one. Absence of a new peak in the chromatograms was used to indicate that no reaction happened between the drug and excipients and so compatible.

2.7. Optimization of hydroxyurea dry syrup

Different formulas were produced by using robustness approach with targeted concentration **of** HU of 100mg/5ml in the final product upon reconstitution because this is seemed to be the most flexible dosage to pediatric population (4). The primary package of the medication was desired to be amber colored glass bottle because the photolysis study of HU is not yet certain, so it's worth to protect the product against photolysis (19). Bottle of 100ml which carried 2gm of the medication equivalent to 20 doses of 100mg/5ml each were prepared. For a laboratory scale preparation, the batch size was made to be 2 bottles equivalent to 4gm of the API. Six trials named Control formula, F1a, F1b, F1c, F1d and F1e were formulated varying excipient ratios with constant quantity of API. Detailed procedures for laboratory scale manufacturing of the named trial formulations F1a to F1d are found in appendix iii. The formulations were then analyzed for conformity with USP specifications. As far as powder for reconstitution is concerned the flowability is a very critical parameter. In the named formulations, the optimum formula was considered as the one with optimum features in all aspect. The final formulation was supposed to have good flow properties, optimal assay, optimal dissolution and with reasonable pH moisture content.

2.7.1. Optimization technique

There are two common approach in optimizing formulation namely robustness method and design of experiment method (36). In this study the robustness method was used whereby, different ratios of excipients with constant quantity of API was tried considering the permissible range in the literature and manufacturers recommendations. All the trials made were dry granulated powder or powder blend rather than granulated powder. The basis of robustness approach is trial and error in varying variables until most optimum formulation is obtained. After obtaining the optimal formulation, the API and rest of the excipients were kept constant except Aerosil. The impact of aerosil on flowability of the formulation was then studied. The six trials formulated are shown in Table 1.

Table 1. Formula for each formulation.

S/N	**Ingredients**	**Control**	**F1A**	**F1B**	**F1C**	**F1D**	**F1E**
1	Hydroxyurea	4gm	4gm	4gm	4gm	4gm	4gm
2	Xanthum gum	0.2gm	0.2gm	0.2gm	0.2gm	0.2gm	0.2gm
3	Aerosil 200	-(0%)	0.0684 (0.5%)	0.2072(1.5%)	0.278(2.0%)	0.348(2.5%)	0.4206(3.0%)
4	Sodium citrate	3.0gm	3.0gm	3.0gm	3.0gm	3.0gm	3.0gm
5	Sodium benzoate	1.4g	1.4gm	1.4gm	1.4gm	1.4gm	1.4gm
6	Mannitol	5.0gm	5.0gm	5.0gm	5.0gm	5.0gm	5.0gm
7	Total	13.6gm	13.6684	13.8072	13.878	13.948	14.0206

Six different formulations were produced by varying the proportions of excipients while maintain constant quantity of hydroxyurea.

2.7.2. The impact of aerosil on the flowability of hydroxyurea dry syrup

It is very clear that the glidants normally improve the flowability of the powder blend but to what extent it is not very clear and that varies depending on the type of glidants and how it interacts with other ingredients in the formulation. In this study all other excipients in the dry syrup were kept constant and the percentage of aerosil 200 was varied in the range proposed by the manufacturer 0.5%w/w to 3.0 % (39).The flow properties were then investigated which included angle of repose, bulk density, tapped, density compressibility index and Hausner's ratio. The investigation involved both control formulation which does not contain aerosil and the rest of the formulations.

2.8. To investigate the physicochemical properties of formulated dry syrup

The four major properties of dry syrup which were investigated included the flowability, assay and dissolution and moisture content. The protocols used were as follows

2.8.1. Powder flowability study protocol

The flow properties which were studied include the following

i. Angle of repose was calculated by considering the height and radius of fixed mass of powder heap and then find out its tangent angle as described by Shah et al 2008 (26). Exactly 5gm of the powder was weighed and placed at the funnel of internal diameter 10mm and internal height of the funnel of 111mm, the funnel was placed at height of 4cm above the base or paper. Then powder was allowed to flow through the funnel to form a heap of powder where the height and diameter of the heap were measured, angle of repose was given as a tangent of an angle between the height and the base. As shown below

   θ =1/ tan (H/0.5D) or θ = 1/tan (H/R) whereby H=Height, D=diameter, R=Radius

ii. Bulk density and tapped density was determined as per method described by Bangar et al 2019 .Bulk density was calculated by considering the total mass of the powder over the bulk volume before tapping (40). A powdered drug of 20gm was weighed and placed in 50ml measuring cylinder then the volume occupied by this powder was measured. Bulk density was given as by the formula below

   Bulk density=mass of powdered drug/bulk volume of powdered drug

iii. Tapped density measurement involved weighing of mass of the powder and its tapped volume, therefore 20gm of powdered drug was placed in 250ml measuring cylinder then the measuring cylinder was placed in the tapping testing machine and allowed 50 tapings and finally the tapped volume was measured. The same procedures was done at 500 and 1250 tapings (41). The tapped density was be given by the formula below,

   Tapped density =mass of the powdered drug/tapped volume of the drug.

iv. Compressibility index is another measure of the powder flow properties which is more precise than angle of repose and is given by the formula below

Compressibility index=tapped density-bulk density/tapped density

v. Hausner's ratio is the measure of flow properties which goes together with compressibility index and was calculated as shown below

Hausner ratio=tapped density/bulk density.

2.8.2. Assay of formulated dry syrup

The formulated dry syrup of HU was quantified by using a validated HPLC method described by Plusce and Yuan with minor modification (23) . The calibration curve used to quantify HU in the formulation was developed by using seven serial dilutions whose concentration ranged from 0.03125mg/ml to 2mg/ml as described in appendix i.

An assay samples were prepared from finished pharmaceutical product (FPP). The weight of FPP equivalent to one administered dose (100mg) was weighed for each formulation then dissolved in 10ml volumetric flask to make ideal concentration of 10 mg/ml. 5mls of this solution was drawn and placed in 200ml volumetric flask followed by addition of uracil as IS and diluting to volume to make final concentration of uracil as 0.25mg/ml. Formulations were considered as passing the test if the percentage was within the range of 90 to 110% as per USP (24). The HPLC was run in triplicate to avoid errors and results by chances.

2.8.3. In vitro drug dissolution of hydroxyurea dry syrup

The drug release test particularly dissolution profile was done in accordance to USP monograph (30) where type 2 apparatus /paddle was used at 50 rpm, the dissolution media was 900ml of purified water equilibrated at 37±0.5°C.The weight of FPP equivalent to the 100 mg of API which is a dose to be administered was weighed and placed in the dissolution vessels 1 to 6, the vessel number 8 had 100 mg of pure API as comparator (37) .Note that currently there is no comparator for HU dry syrup because such formulation does not exist so pure API had to be used. The UV detector was set at 214nm (22) and after every 5 minutes the 5mls of the media in which the drug dissolved was drawn and replaced automatically. The quantity of Hydroxyurea was quantified directly by HPLC method as described by Plunks and Yuan et al (23). The formulation was required to release more than 85% of the drug within 30 minutes. Finally, the plot of percentage of medication released against time was drawn. The run was done in triplicate manner to reduce errors.

2.8.4. Moisture content determination

The moisture content was determined by Loss on drying (LOD) method. Loss on drying is required for determination of quantity of water and other volatile substance accompanied by the solid substance or powdered substance. In the dry syrup formulations of hydroxyurea, this parameter was done in accordance to USP monograph number 731.Because HU dry syrup contains more than one ingredient its worth to show the LOD method for each and every ingredient as per USP monographs before coming to final method for LOD method for the whole FPP as follows

Hydroxyurea-This should be dried in vacuum at 60° C for 3hrs and it should not lose more than 1% of its weight.

Xanthan gum-This should be dried at 105° C for 2.5hrs and it should not lose more 15% of its weight

D-mannitol- This should be dried at 105°C for 4hrs and it should not lose more than 0.5% of its weight

Aerosil-This should be dried at 105° for 2hrs and it should not lose more than 2.5% of its weight

Sodium citrate dihydrate-This should be dried at 180°C for 18hrs and it should not lose more than 10-13% of its weight.

Sodium benzoate This should be dried at 105 for 4hrs and it should not lose more than 1.5% of its weight.

The LOD for the formulations was conducted in such a way that the HU is not thermally decomposed and Sodium citrate which requires much higher temperature loses all of its volatile matter by using lower temperature of 60 °C but extending drying time until each FPP had constant weight upon successive heating and cooling. The detailed procedures for LOD procedures used are found in appendix iv.

2.9. Evaluation of physicochemical properties of reconstituted syrup

Upon reconstitution two important critical parameters for liquid dosage form were evaluated. Those parameters included Viscosity and pH. Viscosity is important in a sense that it affects the in vitro drug release test while pH indicates product stability (42).Failure to maintain pH within the acceptable range indicates chemical reaction had occurred. It should be noted that sedimentation rate was not checked because all

ingredients except aerosil were water soluble there upon reconstitution we had a solution rather than suspension.

2.9.1. Measurement of pH.

The measurement of pH based on the standard requirement of USP document no 791 together with modification of method described by Akre et al 2012 (24).The quantity of powdered formulation equivalent to one FPP was reconstituted by using distilled water up to 100ml. Then 10mls of this liquid was stored at different temperature 4°C and 25°C for 14 days and after every three days pH readings were taken and recorded.

2.9.2 Rheology of the HU reconstituted dry syrup

Kinexus rotation rheometer with 60mm disc diameter was set to produce a shear at 100rpm. Shearing stress (F) applied as well as rate of shear (G) generated were recorded. The volume of reconstituted HU dry syrup used was 1ml and the entire experiment is detailed in appendix v.

2.9.3. Data analysis plan

Descriptive statistics for quantitative data was used to analyze the values of the variables. Those statistics included measures of central tendency and dispersions. During analysis of qualitative data more than three person were involved in comparisons and making judgement. Both people were expert in the field investigated and had no conflict of interest to bias the judgement. The qualitative data in this case included FTIR spectra chromatograms and rheograms.

CHAPTER THREE

3. RESULTS

3.1.-Hydrolysis kinetic of aqueous HU solution

During calibration curve development, HPLC chromatograms showed the solvent front was retained at 1.5minutes, HU peak at 2.78minutes and Uracil as IS at 6.02 minutes as shown in Figure 4.

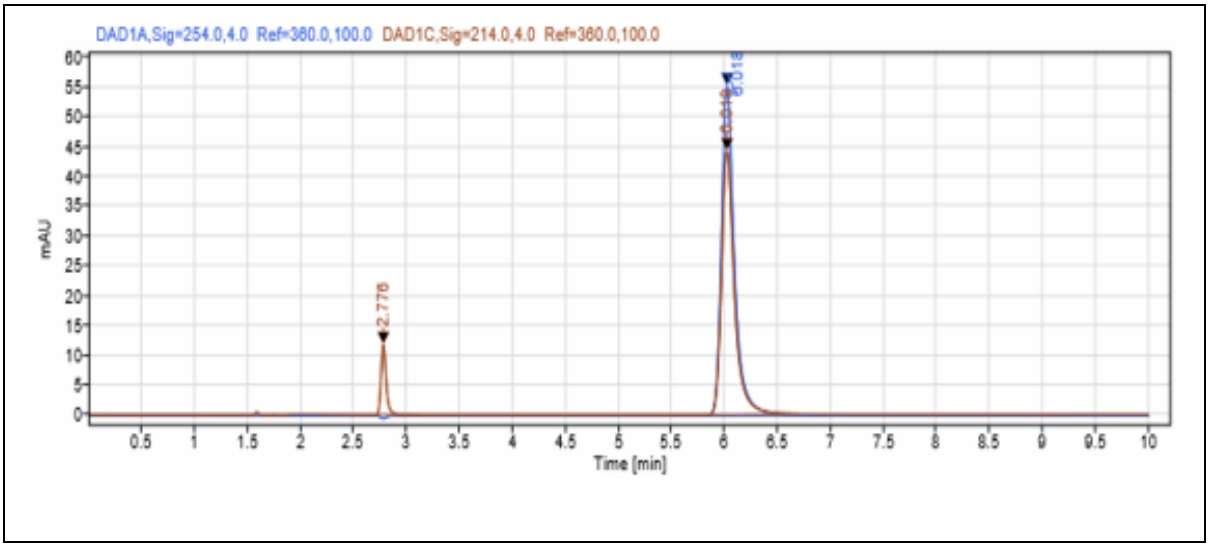


Figure 4: Retention time for hydroxyurea at 2.78minutes and Uracil at 6. 02minutes.

The stock solution of HU kept at elevated temperature of 50 °C demonstrated a linear decrease in concentration with time as by 54th day the concentration was significantly lower.

On the other hand, both stock solutions refrigerated at 4 °C and the one kept at room temperature of 22°C demonstrated stability and concentration of HU did not change with time for the whole study period.

The plot of HU concentration for the stock solution stored at 50°C against time demonstrated zero order kinetics with degradation constant of 0.0367mg/ml/day as shown in Figure 5.

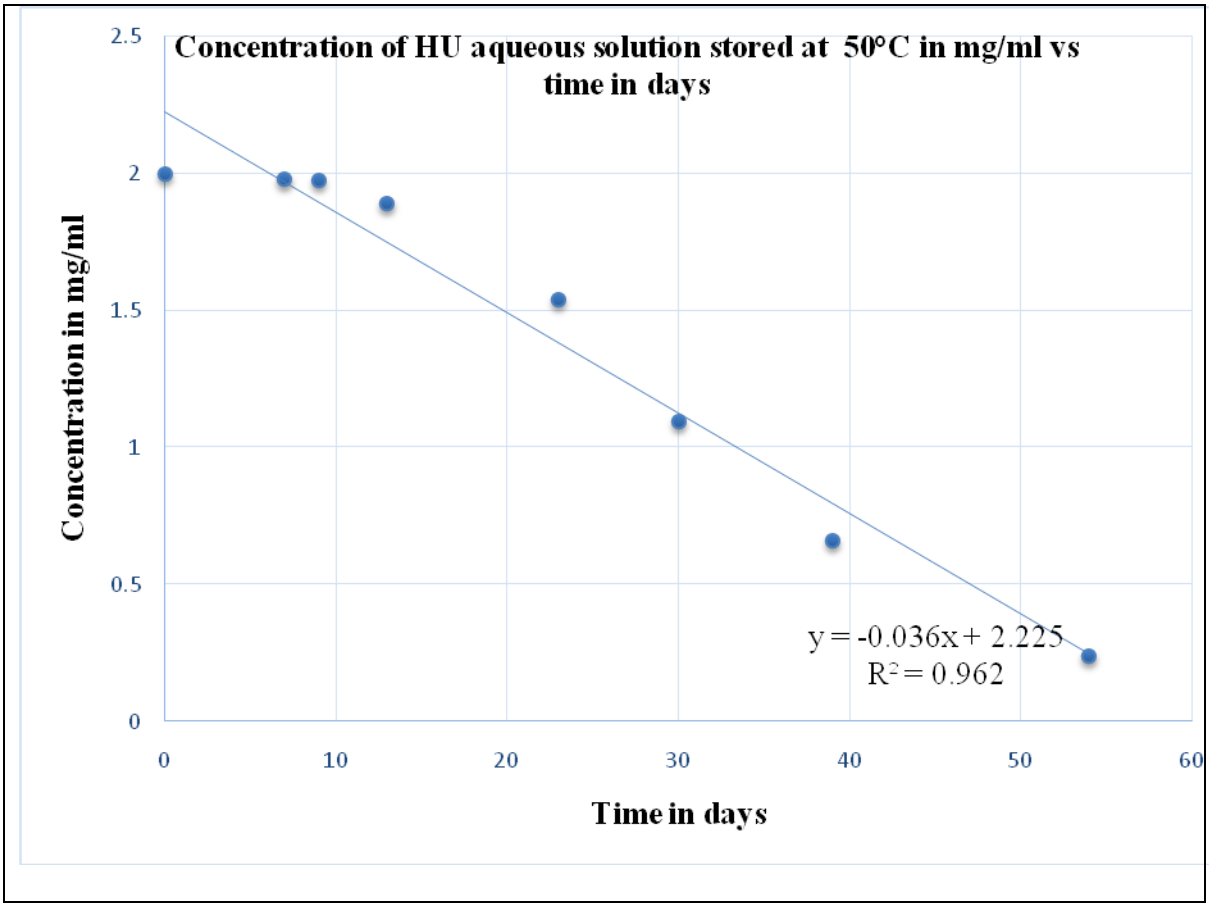
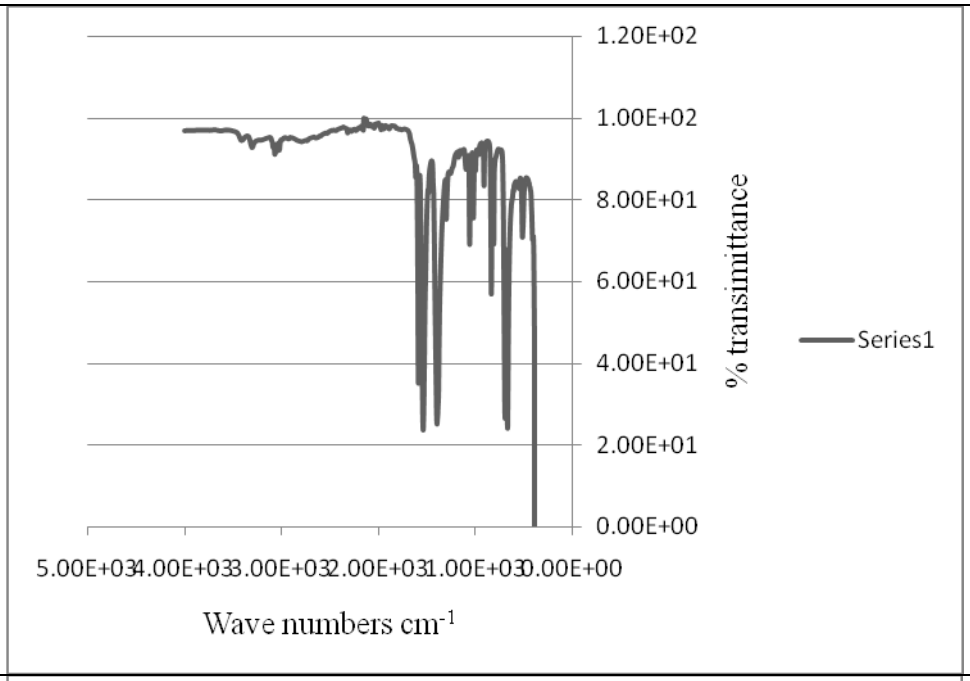
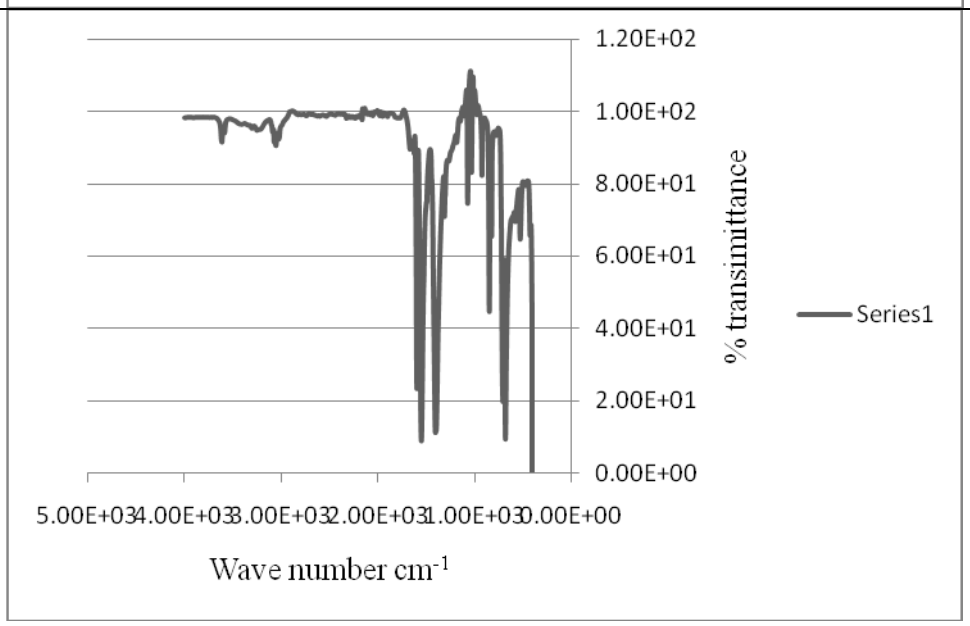


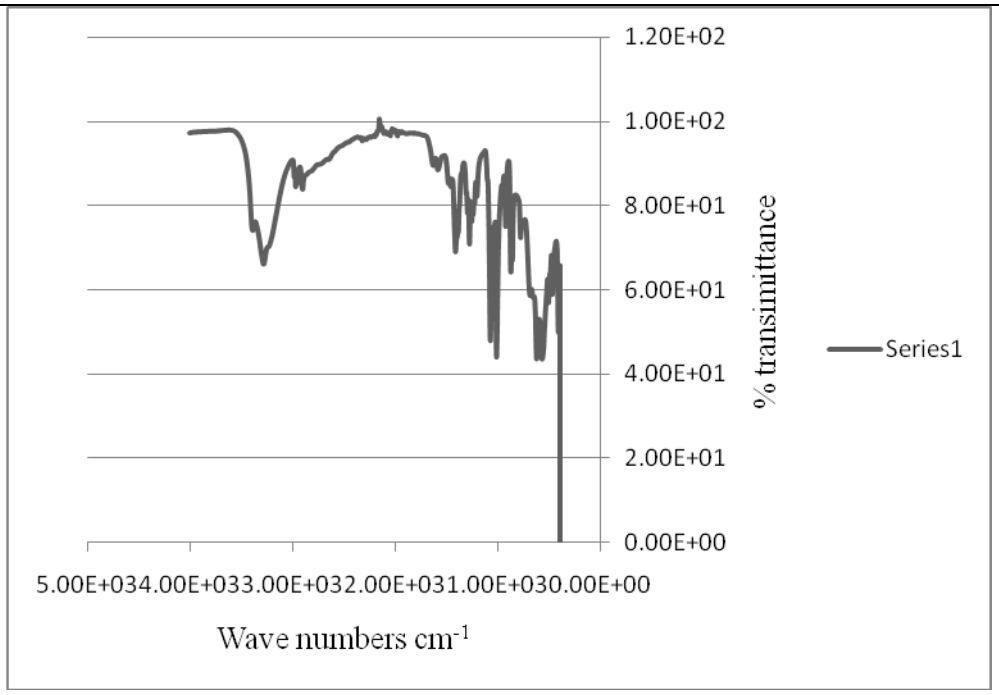
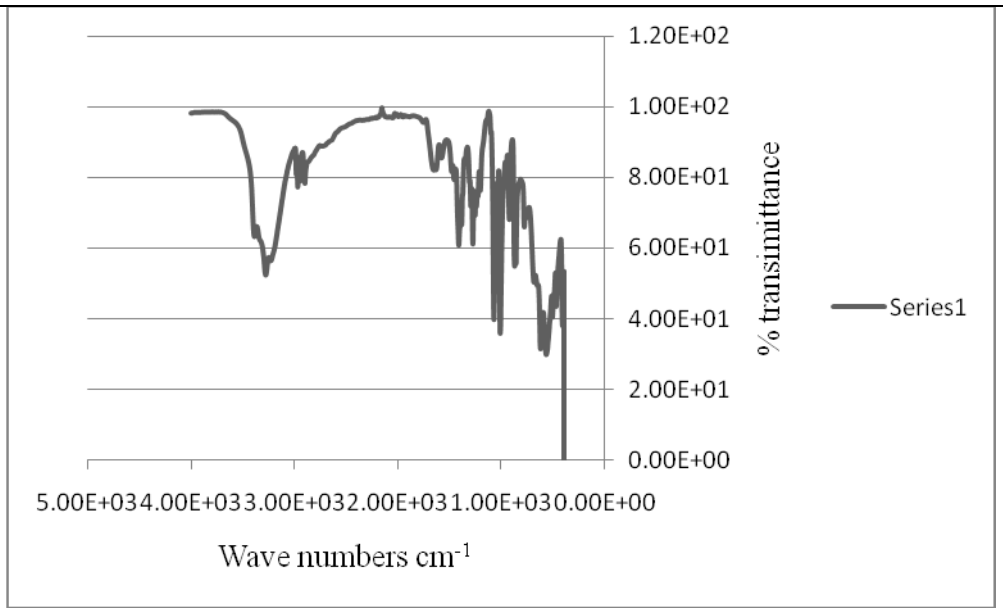
Figure 5: Concentration of hydroxyurea aqueous solution in mg/ml against time in days.

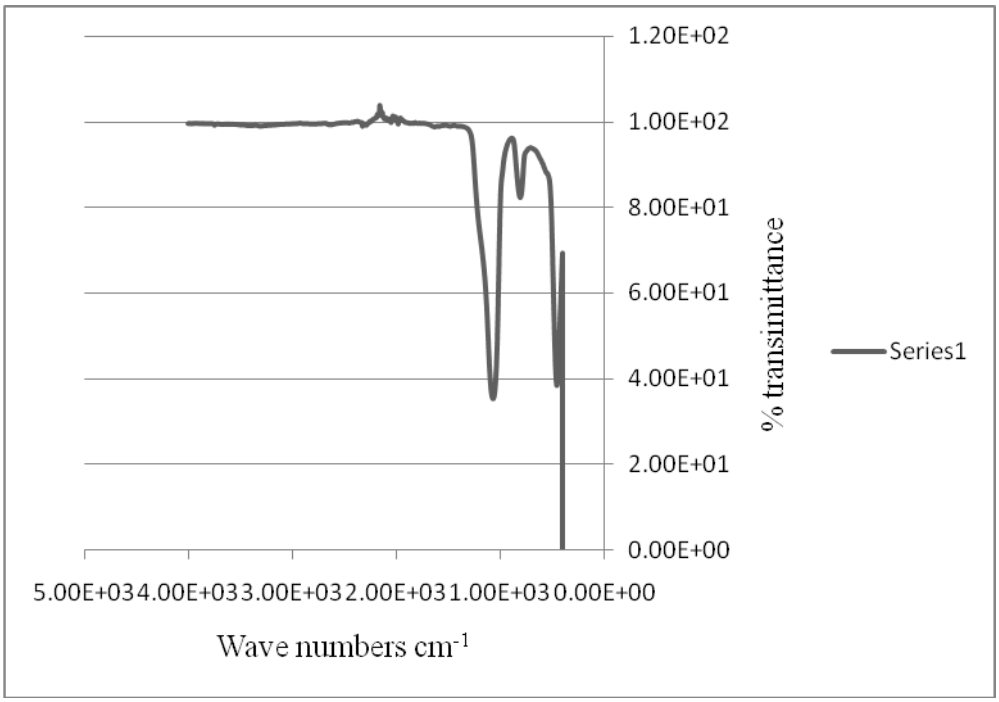
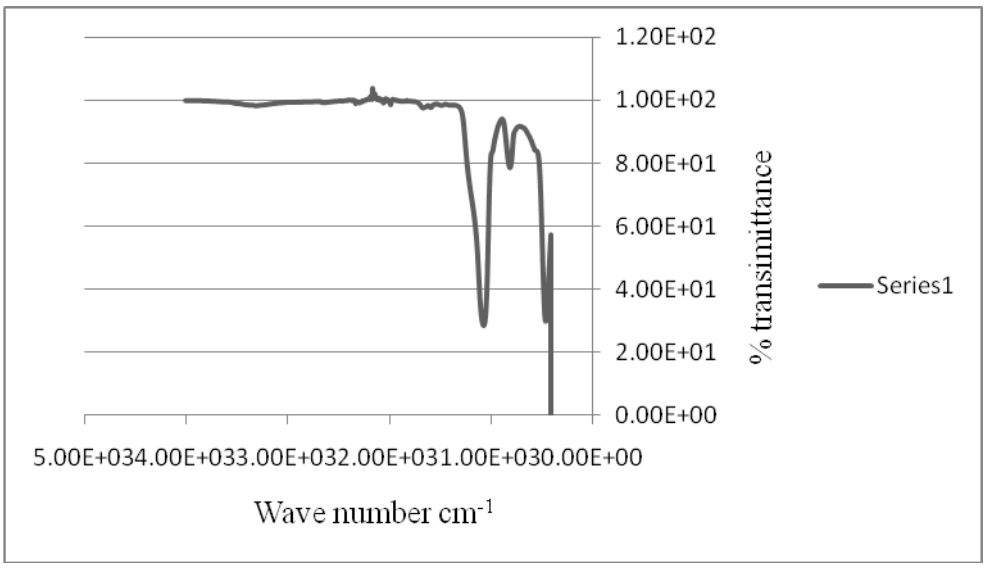
3.2 Chemical Compatibility studies

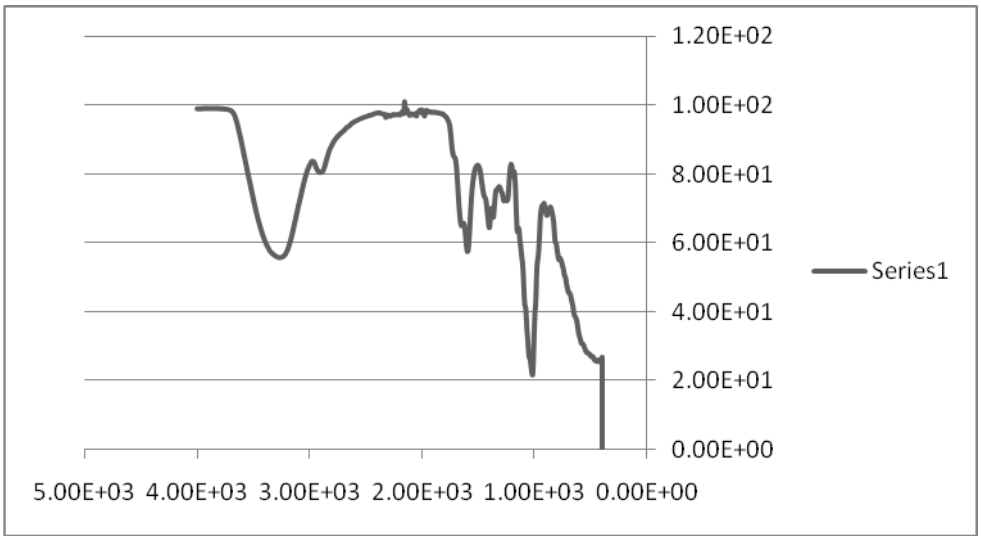
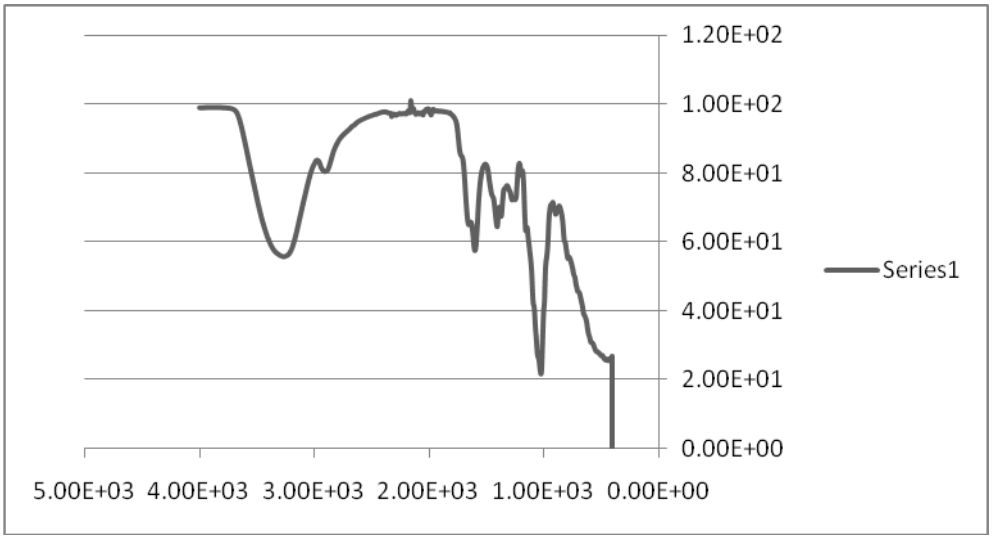
3.2.1. FTIR-Chemical Compatibility between HU and potential excipients

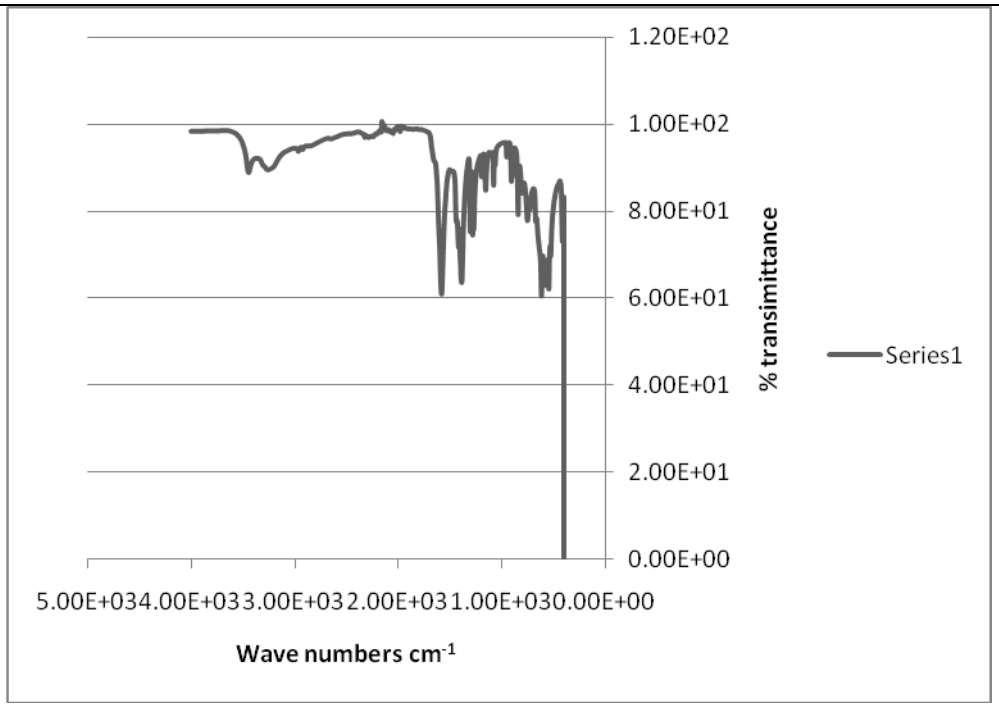
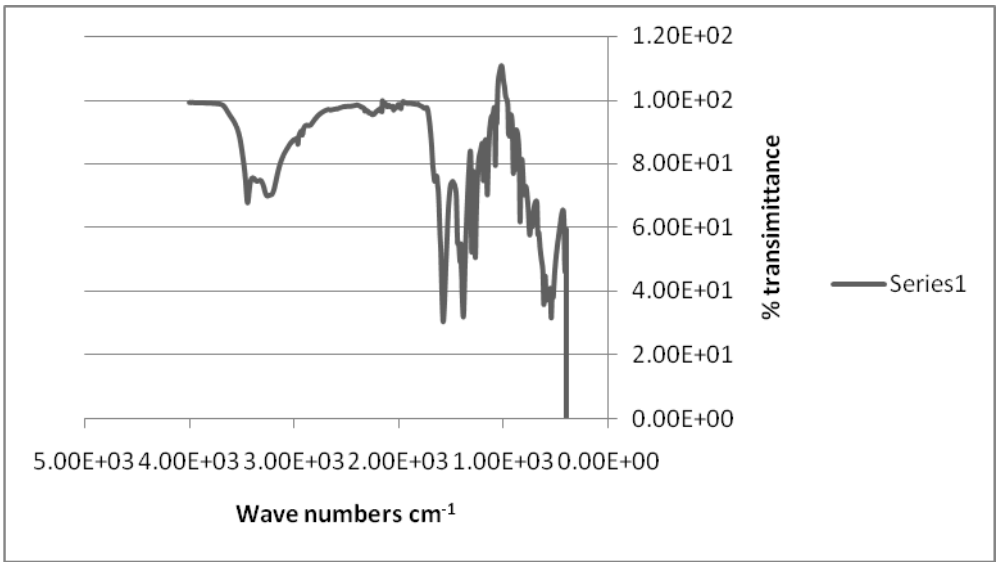
Organoleptic properties analysis indicated no color changes for the all mixtures of API and excipients suggesting absence of chemical reaction hence compatible. Comparison of FTIR spectra produced on day zero and on 28th day were identical indicating the absence of chemical reaction between the API and excipients as shown in Figure 6. Further analysis on functional groups as required by FTIR technique did not show any change in functional group's wave number which suggested absence of reaction between API and excipients as shown in Table 2.

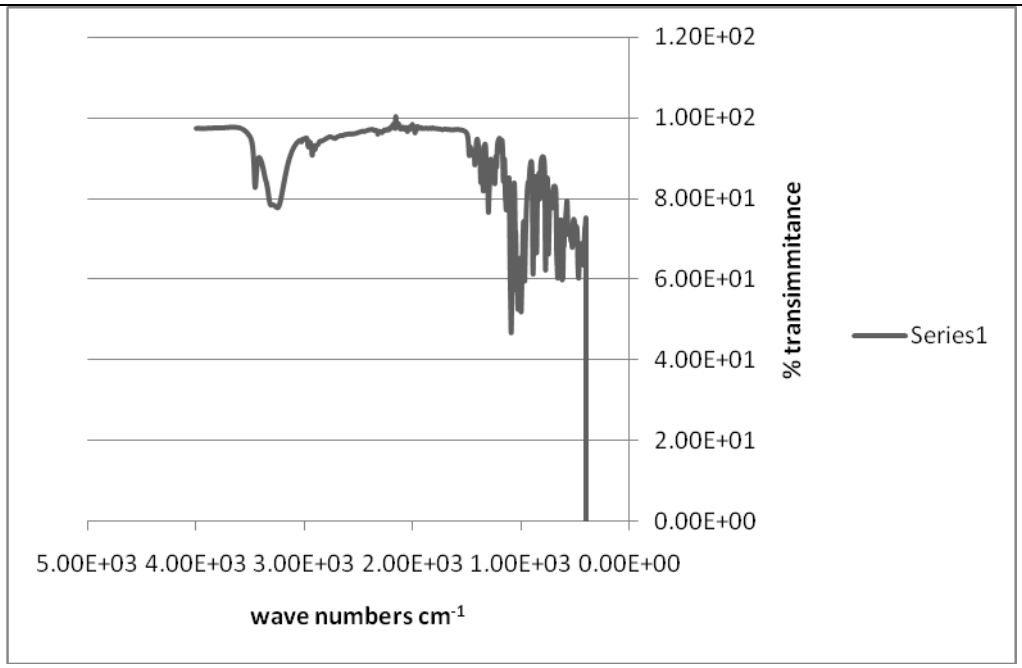
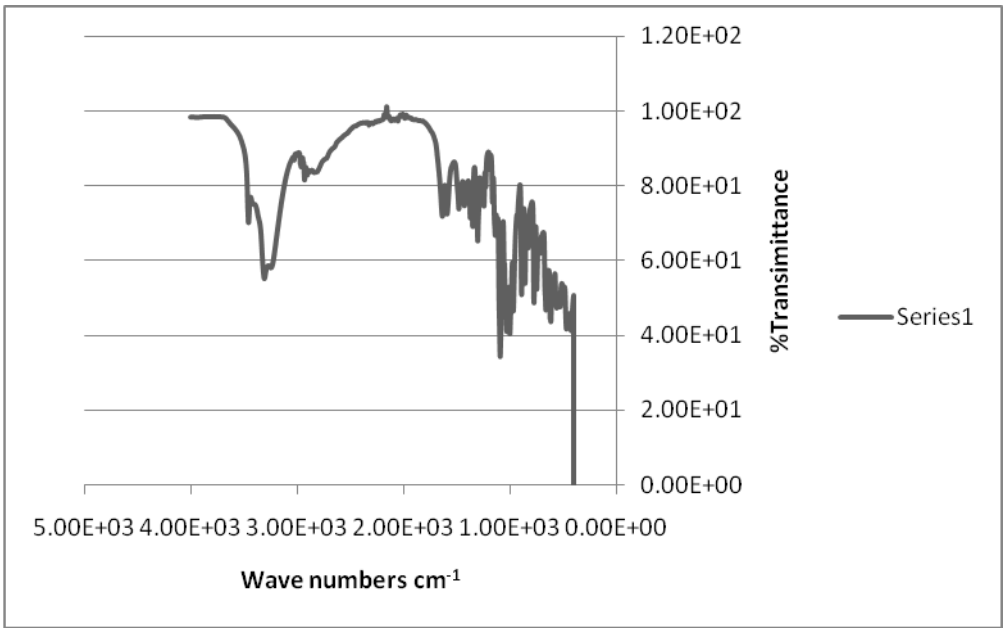
Name of the mixture	Day zero FTIR spectra	Remarks
API+Sodium Benzoate Day zero		Visually two spectra did not differ, they merged peak to peak, so no reaction
Day 28		

API+D-Mannitol Day zero		Visually two spectra did not differ, they merged peak to peak, so no reaction
28th Day		

API+Aerosil Day zero		Visually two spectra did not differ, they merged peak to peak, so no reaction
28th day		

API+Xanthan Day zero		Visually two spectra did not differ, they merged peak to peak, so no reaction
28th Day		
API+Sodium citrate Day zero		Visually two spectra

		did not differ, they merged peak to peak, so no reaction
28th day		
API+Sucralose Day zero		Visually two spectra

		did not differ, they merged peak to peak, so no reaction
28th Day		
API+ALL Day zero		Visually two

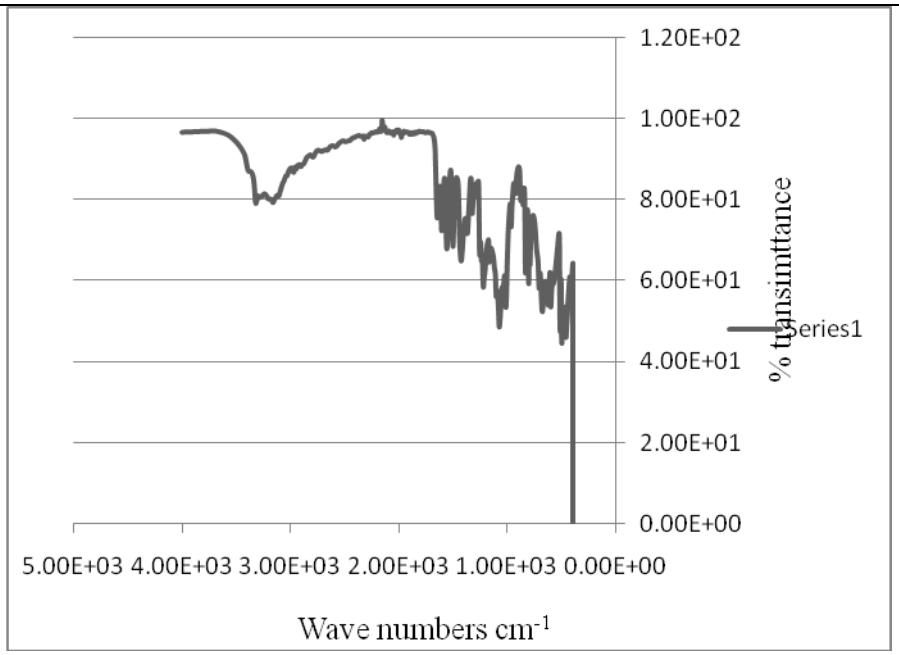
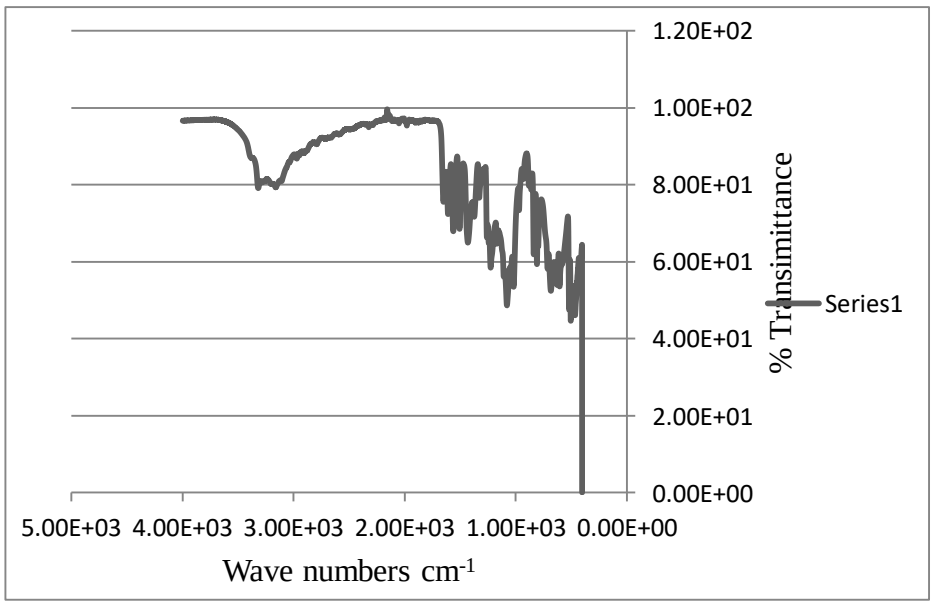
		spectra did not differ, they merged peak to peak, so no reaction
28th Day		

Figure 6.FTIR spectra comparison between day zero and 28th day.

Both spectra were identical hence chemically compatible.

3.2.2. HPLC chromatograms to support FTIR

The HPLC chromatograms acquired on day zero and 28th day were also identical and no new peak was formed. Absence of new peaks in these chromatograms reflect the absence

of chemical reaction between API and potential excipients tested. Even if there might be a possibility of forming UV insensitive molecule, but with dual chemical compatibility study of both FTIR and HPLC such chance is very minimal. The comparisons of HPLC chromatograms is examplified by chromatogram of HU+D- mannitol as shown in Figure 7.

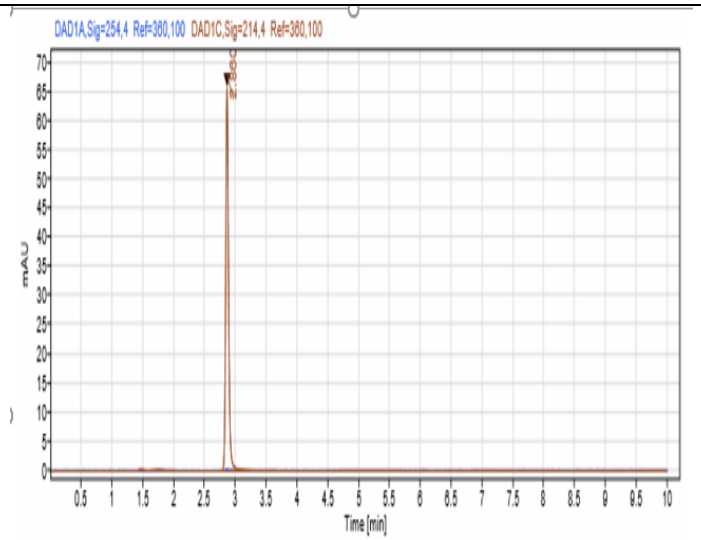
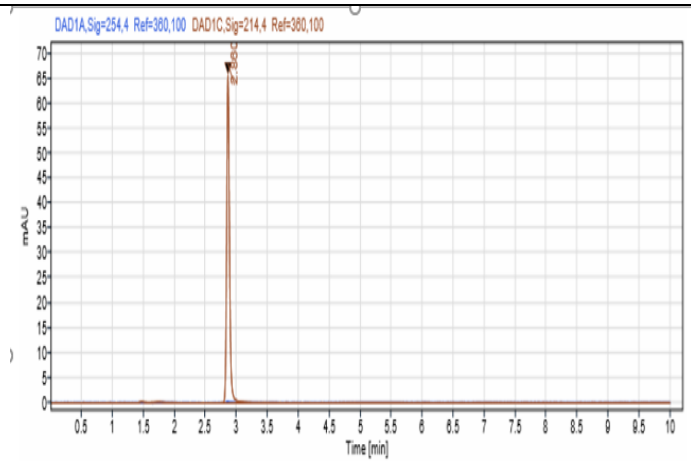
S/N	API+EXCIPIENT	CHROMATOGRAM	REMARKS
1	HU+D-Mannitol-day zero		The principal peak was observed at 2.86 min on day zero and 2.86 on 28th day, extremely smaller peak was observed at 1.5min, suggesting a wave front, therefore similarity in the two chromatograms on different days suggest absence of reactions Note DAD=214nm
	HU+D-Mannitol 28th day		

Figure 7.HPLC-chromatogram for the mixture of hydroxyurea and D-mannitol on day zero and 28th day.

Both chromatograms were identical without formation of new peak and HU was retained at 2.86minutes

Table 2. Comparison of wave numbers of FTIR spectra on day zero and 28th days for pure API as well as different mixtures of API and excipients.

Compound	Functional groups									
	OH, NH_2	=CH-	-CH aliphatic	NH_I	NH_{II}	C=O	C-O	C-N1	C-N2	Remarks
HU-Pure API day zero	3410,3300	-	2810	1580	1480	1630	-	1400	1100	Identity passed
HU-Eu. Ph Ref.Standard	3410,3300	-	2810	1580	1480	1630		1410	1100	
HU+Xanthan gum day zero	3290		2900	1590	1400	1650	1020	1360	1150	No reaction
HU+Xanthan gum 28th day	3290		2900	1590	1400	1650	1020	1370	1140	
HU+D-Mannitol day zero	3280-3390		2900-2970	1580	1420	1660	1020	1390	1280	No reaction
HU+D-Mannitol 28th day	3280-3340		2900-2970	1580	1420	1660	1020	1390	1280	
HU+Sodium Benzoate day zero	3610,3590	3050	-	1550	1400	1590	1070	1310	1180	No reaction
HU+Sodium Benzoate 28th day	3610,3590	3050		1550	1390	1590	1070	1310	1180	
HU+Sodium citrate day zero	3440,3210		2960	1580	1440	1660	1080	1380	1190	No reaction
HU+Sodium citrate 28th day	3440,3240		2960	1580	1440	1660	1080	1380	1190	
HU+Aerosil day zero	-	-	-	-	-	-	-	-	-	No reaction
HU+Aerosil 28th day	-	-	-	-	-			-	-	
HU+Sucralose day zero	3450,3300-3230	-	2930	1590	1480	1630	1090	1350	1110	No reaction
HU+Sucralose 28th day	3455,3310-3240	-	2930	1590	1480	1630	1090	1370	1090	
H-+ALL EXCIPIENTS day zero	3150-3320			1610	1430-1510	1650		1370	1220	No reaction
HU +ALL EXCIPIENTS 28th day	3160-3320			1610	1430-1500	1650	1020	1370	1220	

The similarity in wave number between the mixture observed on day zero and 28th day proved that the API and all excipients had no reaction hence they were compatible.

3.3. Formulation optimization

The most optimized formulation was considered as the one with most optimal physicochemical features. The important physicochemical features were flowability, assay, dissolution, pH, viscosity and desired moisture content as a function of LOD of the formulation. The results for those features are as shown in Table 3.Flowability parameters. judgements were based on table found in appendix vi.

Table 3. Physicochemical features of all formulations for comparison purpose.

S/N	Formulation	1.Flowability					2.Assay	Dissolution	PH at RT	Viscosity	Overall-Remarks
		A. R	B. D	T. D	C.I	H. R					
1	Control	25.24	0.823	0.98	16.05	0.84	99.60%	102.4	7.66-7.54	Pseudoplastic flow	All the formulations passed the test, however the one with optimum features for all test will be considered as most optimized formulation
2	F1a	24.7	0.823	0.952	13.58	0.98	104.80%	89.3	7.73-7.43	Pseudoplastic flow	
3	F1b	24.42	0.83	0.935	11.2	0.88	105.20%	92.9	7.66-7.3	Pseudoplastic flow	
4	F1c	26.22	0.826	0.935	11.57	0.88	113.20%	104.8	7.57-7.3	Pseudoplastic flow	
5	F1d	24.907	0.80	0.926	13.6	0.86	100%	94.05	7.57-7.4	Pseudoplastic flow	
6	F1e	25.333	0.704	0.8	11.97	0.88	106%	98.9	7.57-7.41	Pseudoplastic flow	
7.	Remarks	A.R was excellent in all except F1C had good	Less air accumulation	Less air accumulation	CI was excellent in all	HR was excellent in all	Range 95-105%	Requirement 85% and above in 30min	Range 5-8	Desired flow type is pseudoplastic flow	

All formulations passed the specification test but comparison was made to find the best formulation out of all formulations.

KEY

A.R.=Angle of repose

B. D=Bulk density

T. D=Tapped density

H.R.=Hausner’s ratio

Formulation F1d was the best by having lowest total of counts used to rank them as shown in Table 4. Number of counts for each parameter was ranked from 1-6 whereby 1 was the best and 6 was the worst. Total number of counts scored by the formulation was ranked from 6-36 whereby 6 was the best and 36 was the worst.

Table 4. The optimization ranking of all formulations.

Number of counts for each parameter	1.Flowability	2.Assay	3.Dissolution	PH	Viscosity	Total number of counts of formulation	Optimization ranking
1	F1b	FControl	F1d	F1c	Pseudoplastic flow	Fcontrol=10	F1d with 8counts
2	F1a	F1d	F1a	F1d	Pseudoplastic flow	F1a=12	Fcontrol with 10 counts
3	F1d	F1a	F1control	F1e	Pseudoplastic flow	F1b=13	F1a with 12 counts
4	FControl	F1b	F1c	F1b	Pseudoplastic flow	F1c=15	F1b with 13 counts
5	F1e	F1e	F1b	Fcontrol	Pseudoplastic flow	F1d=8	F1c with 15 counts
6	F1c	F1c	F1e	F1a	Pseudoplastic flow	F1e=19	F1e with 19 counts

Formulation F1d was found to be the best formulation by having lowest total number of counts used to rank.

3.4. The impact of aerosil on the flowability of hydroxyurea dry syrup

Different flowability parameters ranged from good to excellent on increasing the concentration of aerosil (Table 5). Although, after maximum concentration of aerosil was achieved the turning point was observed whereby, the further increase in concentration decreased flowability property as shown in Figure 8.

Table 5: Relationship between the concentration of glidant against flowability.

S/N	Formulation	Concentration of aerosil w/w%	Parameters	Remarks
			Angle of repose	
1	Control	0.0	25.24	Excellent
2	F1a	0.5	24.7	Excellent
3	F1b	1.5	24.42	Excellent
4	F1c	2.0	26.22	Excellent
5	F1d	2.5	24.907	Excellent
6	F1e	3.0	25.333	Excellent
	Formulation	Concentration of aerosil w/w%	Bulk density	Remarks
1	Control	0.0	0.823	Good
2	F1a	0.5	0.823	Good
3	F1b	1.5	0.83	Good
4	F1c	2.0	0.826	Good
5	F1d	2.5	0.80	Good
6	F1e	3.0	0.704	Excellent
	Formulation	Concentration of aerosil w/w%	Tapped density at 1250strokes	Remarks
1	Control	0.0	0.98	Easily packed
2	F1a	0.5	0.952	Easily packed
3	F1b	1.5	0.935	Easily packed
4	F1c	2.0	0.935	Easily packed
5	F1d	2.5	0.926	Easily packed
6	F1e	3.0	0.8	Easily packed
	Formulation	Concentration of aerosil w/w%	Compressibility index	Remarks
1	Control	0.0	16.05	Fair
2	F1a	0.5	13.58	Good
3	F1b	1.5	11.2	Good
4	F1c	2.0	11.57	Good
5	F1d	2.5	13.6	Good
6	F1e	3.0	11.97	Good
	Formulation	Concentration of aerosil w/w%	Hausner's ratio	Remarks
1	Control	0.0	0.84	Excellent
2	F1a	0.5	0.98	Excellent
3	F1b	1.5	0.88	Excellent
4	F1c	2.0	0.88	Excellent
5	F1d	2.5	0.86	Excellent
6	F1e	3.0	0.88	Excellent

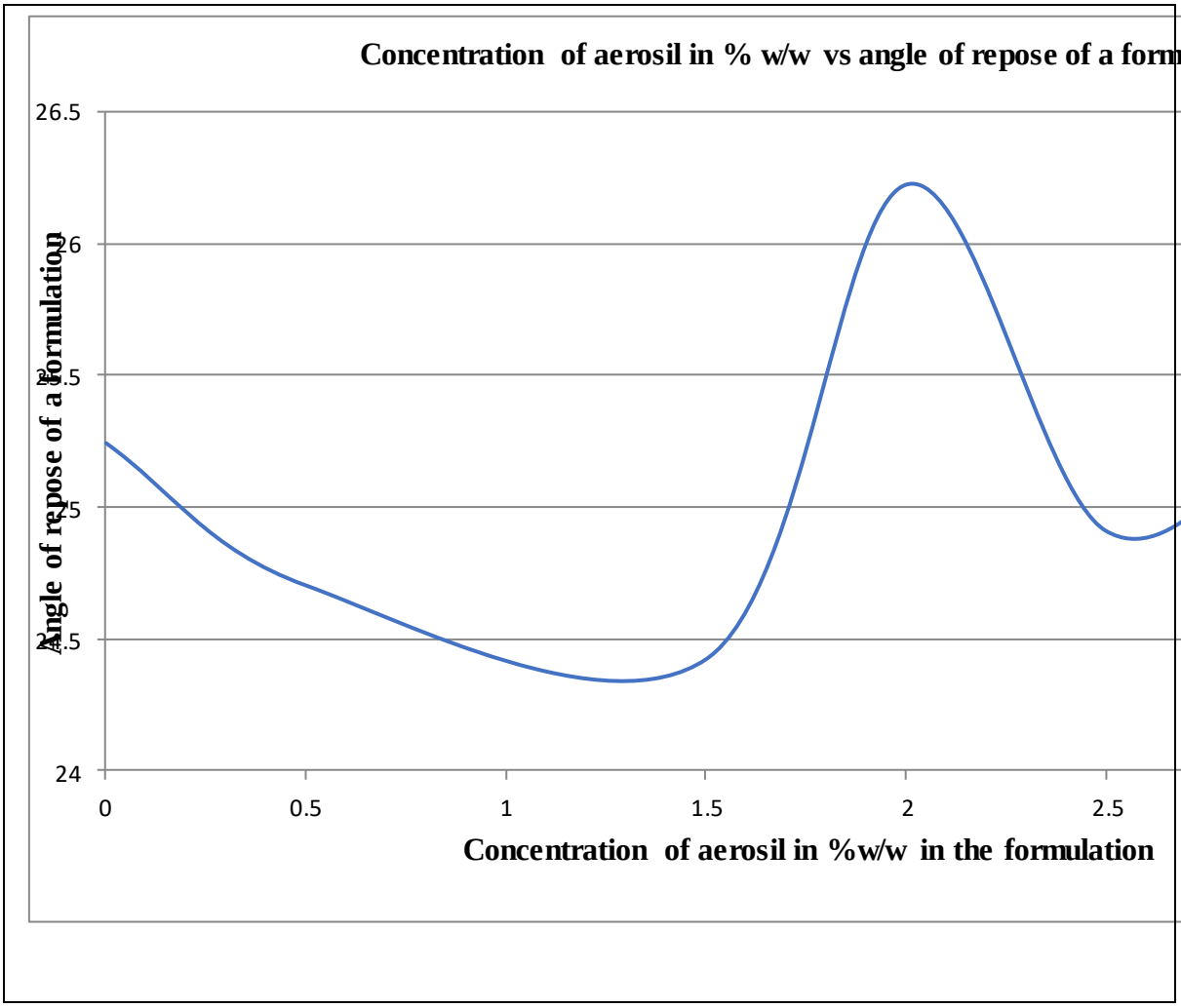


Figure 8.Impact of increasing concentration of aerosil on angle of repose of the formulation.

Turning point was observed at 2%w/w concentration of aerosil when angle of repose starts to decrease upon further increase in aerosil.

3.5. Physicochemical properties of formulated dry syrup and its reconstituted syrup

The physicochemical properties for dry syrup included flowability, assay and dissolution. **On** the hand the physicochemical properties for reconstituted syrup were PH and rheology.

3.5.1. Assay of the formulations

The calibration curve developed showed linearity with equation stated as y = 99.623x + 3.8342 whereby R^2 =0. 99.The equation obtained was used to calculate drug content on each formulation. Results are shown in Table 6.

Table 6.Assay of all the formulations.

Concentration of each formulation as a function of its peak areas from X=(Y-3.834)/99.62					
S/N	Formulations	Peak areas	Conc in mg/ml	Assay =conc/0.25 ×100	Remarks
1	Control form	28.644	0.249	99.60%	Passed
2	F1a	29.905	0.262	104.80%	Passed
3	F1b	30.083	0.263	105.20%	Passed
4	F1c	32.005	0.283	113.20%	fail
5	F1d	28.713	0.25	100%	Passed
6	F1e	30.23	0.265	106%	Passed

Five formulations passed the test except F1c had higher drug content than they acceptable criteria 0f 90-110% as per USP (42).

3.5.2. Dissolution profile for different formulations

The developed calibration curve showed linearity with the equation stated as y = 3E+06x + 68754, the R^2 =0.99. By considering the peak areas (PA) of each formulation the drug released at each time for each formulation were shown in Figure 9.

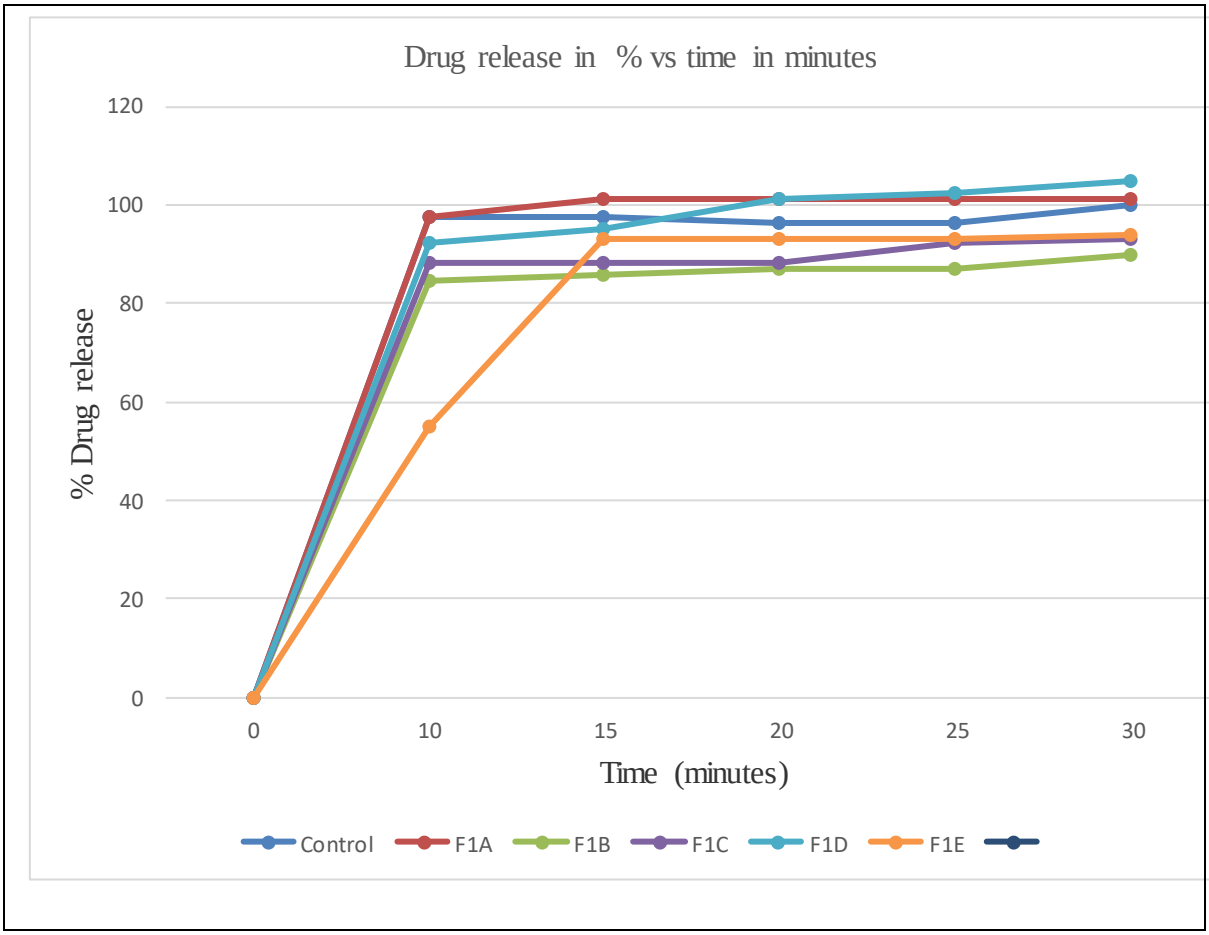


Figure 9: The dissolution profile for all the formulations.

All formulations passed the test by releasing more than 85% within the first 30minutes as required by USP (42).

3.5.3. Evaluation of pH of the reconstituted syrup

The syrups showed a desirable range of pH of 5-8 for the duration of 14 days as shown in **Error! Reference source not found.**.

Table 7. pH of the formulations stored in the refrigerator at 4°c and room temperature of 22-25°c for 14 days after reconstitution.

Stored at 22-25°C	**Day1**	**S/N**	**pH1**	**pH2**	**pH3**	***Av. pH**	***S. d**	**Remarks**
		Fcontrol	7.63	7.63	7.73	7.66	0.04	Pass
		F1a	7.71	7.66	7.66	7.68	0.02	Pass
		F1b	7.61	7.58	7.57	7.59	0.02	Pass
		F1c	7.57	7.58	7.57	7.57	0.00	Pass
		F1d	7.56	7.56	7.55	7.56	0.00	Pass
		F1e	7.54	7.54	7.55	7.54	0.00	Pass
	Day7	Fcontrol	7.47	7.45	7.46	7.46	0.01	Pass
		F1a	7.53	7.51	7.52	7.52	0.01	Pass
		F1b	7.51	7.40	7.50	7.47	0.05	Pass
		F1c	7.39	7.40	7.41	7.40	0.01	Pass
		F1d	7.36	7.36	7.38	7.37	0.01	Pass
		F1e	7.37	7.37	7.36	7.37	0.00	Pass
	Day 14	Fcontrol	7.54	7.53	7.55	7.54	0.01	Pass
		F1a	7.43	7.44	7.42	7.43	0.01	Pass
		F1b	7.30	7.31	7.29	7.30	0.01	Pass
		F1c	7.31	7.30	7.29	7.30	0.01	Pass
		F1d	7.44	7.45	7.43	7.43	0.01	Pass
		F1e	7.41	7.39	7.42	7.41	0.01	Pass
Stored at 4°C	Day1	Fcontrol	7.63	7.67	7.68	7.66	0.02	Pass
		F1a	7.64	7.64	7.64	7.64	0.00	Pass
		F1b	7.58	7.58	7.57	7.58	0.00	Pass
		F1c	7.58	7.60	7.59	7.59	0.01	Pass
		F1d	7.53	7.51	7.51	7.52	0.01	Pass
		F1e	7.57	7.59	7.58	7.58	0.01	Pass
	Day 7	Fcontrol	7.55	7.66	7.57	7.59	0.04	Pass
		F1a	7.58	7.55	7.57	7.57	0.01	Pass
		F1b	7.50	7.50	7.49	7.49	0.00	Pass
		F1c	7.50	7.48	7.49	7.49	0.01	Pass
		F1d	7.54	7.46	7.48	7.49	0.03	Pass
		F1e	7.38	7.48	7.42	7.43	0.03	Pass
	Dy14	Fcontrol	7.89	7.88	7.90	7.89	0.01	Pass
		F1a	7.51	7.49	7.53	7.51	0.01	Pass
		F1b	7.67	7.66	7.68	7.67	0.01	Pass
		F1c	7.42	7.44	7.38	7.41	0.02	Pass
		F1d	7.33	7.36	7.33	7.34	0.01	Pass
		F1e	7.42	7.38	7.43	7.41	0.02	Pass

For the entire period of 14 days the syrups maintain pH within a narrow range of 7.3 to 7.8 hence passed.

3.5.4. Viscosity of the reconstituted syrup

All the formulations had proven pseudoplastic flow which is the most desired flow behavior for pharmaceutical solutions and suspension has shown by the rheograms in Figure 10.

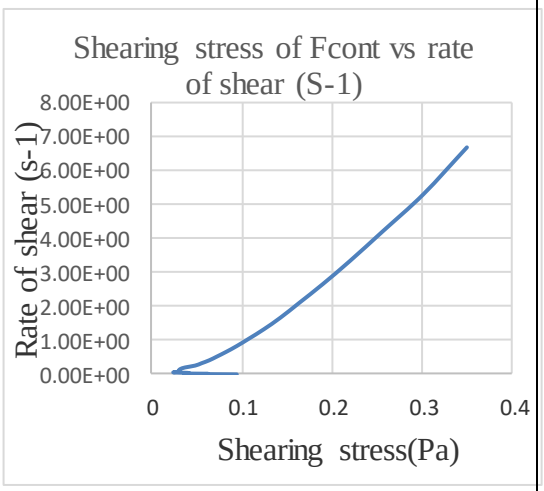
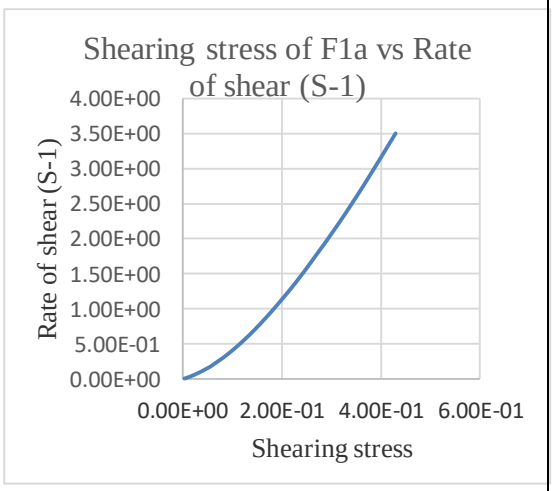
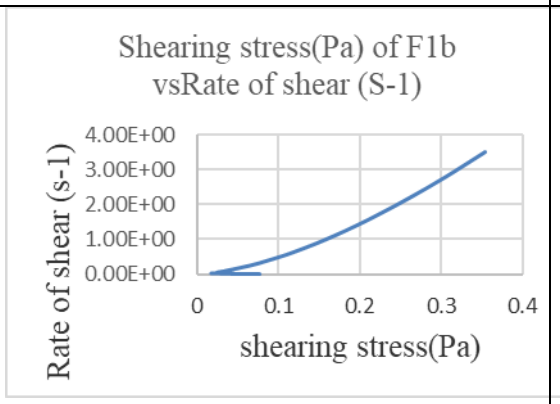
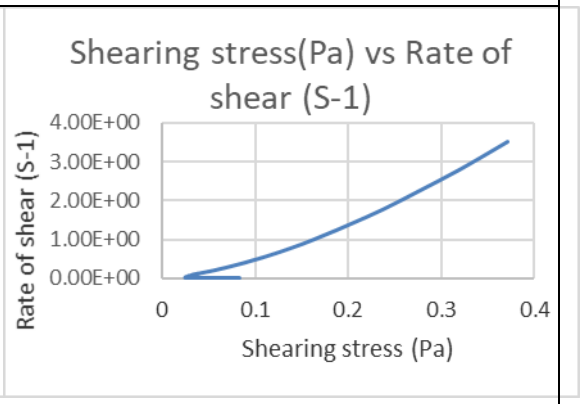
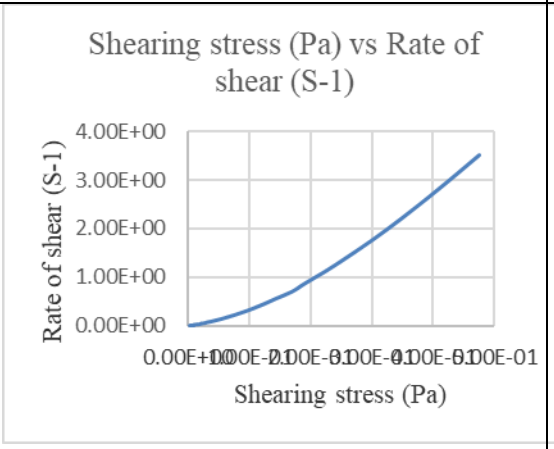
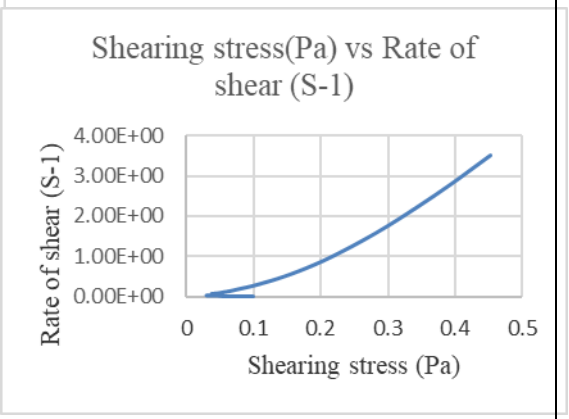
S/N	Fcontrol	F1a
1		
2	F1b	F1c
		
3	F1d	F1e
		

Figure 10: Pseudoplastic rheograms of formulations acquired when the dry syrups were equilibrated at temperature of 22°C.

3.5.5. Moisture content determination

All the formulation has shown a MC of less than 1% which was the desired level for dry syrup especially HU -FPP which has very hygroscopic API as shown in Table 8 .

Table 8.Moisture contents for all formulations obtained by LOD method.

	Wo	WI	WF	Wo-Wf	MC=(Wo-Wf)/Wo x100	Remarks
Fcontrol	3619.4	3618.2	3613.6	5.8	0.160247555	Excellent
F1a	3640.9	3639.2	3639.2	1.7	0.046691752	Excellent
F1b	3617.3	3615.7	3615.1	2.2	0.060818843	Excellent
F1c	3685.4	3684.1	3684.1	1.3	0.035274326	Excellent
F1d	3644.1	3644.1	3643.7	0.4	0.010976647	Excellent
F1e	3664.9	3652.4	3651.7	13.2	0.360173538	Excellent

All formulations had desirable moisture content sufficient to confer stability of the formulation.

CHAPTER FOUR

4. DISCUSSIONS

Hydroxyurea is highly hygroscopic thus its physicochemical stability when prepared as an aqueous solution was a major concern. Even so, formulating hygroscopic substance or aqueous unstable substance into dry syrup has been reported to maintain their physical and chemical stability and remaining stable for 14 days upon reconstitution (37).

In the preformulation study the aqueous solution of the reference HU was stable for three months when stored at room temperature of 22-25°C or upon refrigeration at 4°C. Even so, HU started to underwent hydrolysis after two months of being stored at elevated condition of 50°C. The HU hydrolysis exhibited zero order kinetics as shown in Figure 5. These findings are in agreement to the earlier study conducted by Heeney eta al 2015 (4). In this, study the HU capsule were compounded to make solutions, and maintained the stability up to three months upon refrigeration at 4°C (19). Dry syrup upon reconstitution should be able to maintain physical and chemical stability for at least 14 days when stored either at room temperature or upon refrigeration as per USP pharmacopeia requirements (11). In this study HU hydrolysis kinetic study provided a clue on the stability of the formulated dry that upon reconstitution it can be stable for more than 14 days at room temperature and when refrigerated.

During chemical compatibility studies, the initial spectra and final spectra were similar indicating that all excipients were chemically compatible with the API as shown in Figure 6. The FTIR analysis in the current study is in line with the study conducted by Hussein et al 2015 whereby, both wave numbers and spectra for HU were identical (43). Since API was chemically compatible with all potential excipients, this predicted a potentially chemical stable HU dry syrup formulation.

In formulation optimization, the formula F1d was found to be the most optimum formulation. Though this formulation had proven to be the most optimum formulation nevertheless, during up-scaling some changes may occur which was not observed at laboratory scale (44).

The values of flowability parameters including angle of repose, compressibility index and Hausner's ratio were all in agreement with the USP specifications (45). Powder

flowability is an important parameter for both dry granulated or wet granulated pharmaceutical powders as it may affect filling of powders in primary containers which in turn affects dosage uniformity .Therefore, better flowability predicts better dosage uniformity (46) .

The quantity of drug quantified in these formulations during assay complied to the specifications of USP. The acceptable range of assay is from 90% to 110%, this range of assay actually reflect the uniform distribution of API during mixing (41) .Lower or higher assay value reflects that the API was not uniformly distributed during mixing. The FPP formed from the part of the mix with higher API might have potential to cause toxicity because the dose size will be greater than the labeled strength.

The dissolution profiles exhibited by the all formulations were found to be within the acceptable limit of USP (41). The formulation is required to release more than 85% of its drug within first 30 minutes as far as USP is concerned (41). Ability of the formulation to pass this test implies that the FPP upon administration will release sufficient medication at the absorption site (30).

The reconstituted dry syrup had demonstrated a narrow change in pH for the study period of 14 days. The required pH range for the dry syrup is between 5-8 but in this study pH was maintained between 7.3 -7.8 (41). This pH analysis study is in line with the study conducted by Harshada et al 2012 that indicated that a dry syrup ability to maintain narrow pH range reflects the chemical stability of the reconstituted syrup (24). Ability of a dry syrup to maintain the pH for the duration of 14 days upon reconstitution indicates absence of either hydrolysis or degradation of API by any mechanism (25).

Viscosity is a very important parameter as far as liquid dosage form is concerned as it affects the drug release from FPP into GI fluid. Very high viscosity creates a barrier or resistance through which a drug or API can permeate to reach biological membrane. Upon reconstitution all formulations had exhibited pseudoplastic flow which is a desired viscosity property for either pharmaceutical suspension or solution. The flow property exhibited by these formulations are in line with the study conducted by Bila et al 2000 where xanthan polymer exhibited pseudoplastic in a number of formulations (28). The

shear thinning property of these formulations allows a decrease in viscosity upon shaking so that the drug can be ready to be released upon administration.

All the formulations had demonstrated a MC of less than 1% which is within the required range of pharmaceutical powders that should be not greater than 7 % (26).Higher values of MC affects chemical stability, powder flowability, cause caking of powder as well as increase the chance of microbial growth in the formulation. For the formulations with hygroscopic API such as HU the lower the MC value the more stable formulation (47).

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The HU dry syrup formulation was successfully developed and optimized and formulation F1d was found to be the best among all formulations. This successfully developed and optimized HU dry syrup has a potential to alleviate the dosage challenges which are currently faced by pediatric population. This formulation has a capability to individualize the dose of HU basing on child weight due its flexibility upon reconstitution. Dosage individualization can reduce the number of ADR accompanied with the use of HU and improve the quality of life of SCD children. The composition and ratio of each ingredient in the optimized formula are shown in Table 9.

Table 9.The formula of the most optimal formulation namely F1d

S/N	**Ingredients**	**Quantity in gm**	**Function in the formula**
1	Hydroxyurea	4gm	API-exerting therapeutic value of the formulation
2	Xanthum gum	0.2gm	Thickening agent
3	Aerosil 200	0.348	Glidant and anticaking agent
4	Sodium citrate	3.0gm	PH modifier
5	Sodium benzoate	1.4g	Preservative
6	Mannitol	5.0gm	Sweetening agent
7	Total	13.6gm	

5.2 Recommendations

5.2.1. Stability studies to establish shelf life

Both real time and accelerated stability studies are recommended so as to establish the shelf life and fully characterize all the formulations developed.

5.2.2. Cost effective analysis for production of each formulation

Each formulation developed had different ratio of excipient used hence quantity of excipients differs too. The differences in the quantity of excipients used may have cost implication. Therefore, cost analysis for each formulation will tell the economic feasibility of that formulation to be developed in Sub-Saharan Africa.

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APPENDICES

Appendix i: Protocol for calibration curve development

a) Preparation of Internal standard to concentration of 0.25mg/ml. Uracil powder USP was dissolved in distilled water to make the final concentration of 0.25mg/ml.
b) Preparation of stock solution of hydroxyurea reference standard to a concentration of 2mg/ml. Hydroxyurea reference standard powder was dissolved in distilled water to make a final concentration of 2mg/ml.
c) Preparation of standard solution and their dilutions for establishing calibration curve
   The stock solution was diluted by six serial dilutions A to F. Then constant quantity of IS was added in order to have a concentration range between 2mg/ml and 0.015625mg/ml of HU and constant quantity of internal standard in all dilutions.
d) All solutions were filtered in the 0.45 microns filter and poured in vials. The sample A to F were then ran on HPLC to get area under the curve (AUC), the plot of AUC against Concentration was studied.

Appendix ii-Protocol for doing quantification of HU in kinetic study

Apparatus needed were 10mls volumetric flask, nine HPLC vials in which three was labelled as X for refrigeration, three other vials labelled Y for room temperature and last three labelled as Z for storage at extreme condition of 50℃ and the relative humidity higher than 75%.

a) Preparation of stock solution and respective storage conditions
   Hydroxyurea RS- powder was dissolved in distilled water to make final concentration of 2mg/ml. The solution was filtered by 0.45microns filter and placed in HPLC vials labeled as X, Y and Z and placed at different conditions of temperature and humidity. HPLC vials labeled X was placed in the refrigerator maintained at 4˚C while Y at room temperature varying between 20-24˚C and Z at 50˚C and RH greater than 75%
b) HPLC quantification of concentration
   The HPLC was ran in a triplicate fashion so as to make sure nothing happens by chance. Substituting or relating the AUC and the linear equation from the calibration curve the concentration at any time was obtained. Degradation was supposed to be observed by formation of new peaks and decrease in concentration from the original concentration. The HPLC was run for number of days until sufficient points were met

for plotting and decrease in concentration was more significant. A plot of concentration against time was used describe the nature of degradation kinetics.

Appendix iii: Laboratory scale manufacturing of HU dry syrup.

The manufacturing procedure were as follows

a) All ingredients were weighed and placed in separate containers.
b) Initially half of the quantity of aerosil was placed in the manufacturing vessel to prevent other ingredient form sticking to surface of production vessel.
c) The accurately weighed quantity of sodium benzoate was added in the production vessel.
d) The accurately weighed quantity of Hydroxyurea was added to the previous mixture.
e) The accurately weighed quantity of Xanthan gum was added in the previous mixture.
f) The remaining half of aerosil was added in the previous mixture.
g) The accurate weighed quantity of D-mannitol was placed in the previous mixture.
h) Finally, accurate weighed quantity of sodium citrate was added in the mixture, then the production vessel was mounted on the rotor and allowed to rotate for 12 hrs., note that 12hrs was established as optimum mixing time for this type of cylindrical container because of its very smaller rotation speed.
i) Weigh of powder blend weight equivalent to 2gm of API was weighed and placed in the
   100ml amber colored glass bottle.
j) The FPP was then subjected to evaluation test as shown in the next section.
k) The quantity of ingredient weighed for each batch were as shown in the table below.

Appendix iv. Protocol for the determination of moisture content of all formulations

a) Six labeled aluminum plates Fcontrol, F1a to F1e were prepared and dried in oven at 100°C for 30minutes, followed by cooling in a desiccator.
b) The weights of aluminium plates were taken to the precision of two decimal places after recording the weight of plates, the balance was tarred and 2000mg of each formulation was weighed then evenly distributed to a depth less than 5mm.
c) The weighed formulations were placed in oven at 60°C for 54hrs (i.e. 180°c/60°c=3, so 3×18hrs =54hrs), after 54hrs the formulations were cooled in a desiccator weight of each formulation (final weight –weight of plate) then the formulations were subjected

to drying again at 60°C for 4hrs after every 4hrs checking and recording the weight until constant weight was reached.

d) The LOD for each formulation was given by the formula below

LOD= (Initial Weight-Final constant weight/Initial weight) ×100

Appendix v. Rheological study of the HU reconstituted dry syrup.

A rotating disc rheometer branded as Kinexus rheometer operated with a Malvern software dictated the whole procedure. The software required specific disc diameter and specific volume of reconstituted liquid. The procedures were as follows

a) The disc of 60mm diameter was inserted in its place inside viscometer.
b) After cleaning of both disc and shearing surface the zero gaping was done.
c) The liquid sample immediately shaken on vortex generator was prepared for dropping on surface.
d) 1ml of the liquid was placed in the shearing surface and the disc was brought to contact followed by treaming sideways and covering the surface.
e) The temperature was allowed to equilibrate with the programmed temperature and starting measuring viscosity.
f) The results of viscosity against shearing rate was produced and analyzed to tell the viscosity and the flow type of each formulation.

Appendix vi. Flowability table Showing the range of flowability parameters used to make judgement.

SN	Parameter	Range	Interpretation
1.	Angle of repose	15-30	Excellent
		31-35	Good
		36-40	Fair
		40-45	Passable
		46-55	Poor
		56-65	Very poor
		Above 66	Very, very poor
2	Compressibility index	Less than 10	Excellent
		11-15	Good
		16-20	Fair
		21-25	Passable
		26-31	Poor
		32-37	Very poor
		Above 38	Very, very poor
3.	Hausner's ratio	1.00-1.11	Excellent
		1.12-1.18	Good
		1.19-1.25	Fair
		1.26-1.34	Passable
		1.35-1.45	Poor
		1.46-1.59	Very poor
		Above 1,60	Very, very poor

This table was adapted from United States Pharmacopoeia document number 1174 Powder Flow, page 618 of PF 28.