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Formulation Development and Evaluation of Hydroxyurea Dry Syrup for the Management of Pediatric Patients with Sickle Cell Disease in Tanzania

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Hydroxyurea (HU) is the drug of choice for the management of sickle cell disease but the available dosage form exists as a 500 mg capsule, which is not appropriate for pediatrics whose dosing requirements are 20 mg/kg. The current practice of compounding is prone to dose errors and contamination. Also, shortage of compounding laboratories in hospitals in the developing countries is a major issue. This study aimed at investigating the stability of HU in aqueous solution followed by formulation and evaluation of its dry syrup. Stability of HU aqueous solution was investigated and subsequently dry syrups formulated. They were evaluated for flowability, assay, dissolution, moisture content, rheology and pH. The formulated dry syrups complied with the United States Pharmacopeia (USP) specifications for stability, angle of repose (24-25°), assay (90-110%), dissolution (more than 85% in the first 30 minutes), shear thinning and pH (7.3). HU dry syrup was successfully developed, optimized and found to comply with USP specifications.

Key words: Dry syrup, Formulation development, Hydrolysis kinetics, Hydroxyurea, Sickle cell disease, pediatric.

INTRODUCTION

Sickle cell disease (SCD) is a genetic disease affecting largely pediatrics especially those under five years of age [1]. Worldwide, about 300,000 children are born with SCD per year, of which 75% are born in Africa [2]. Tanzania has one of the highest annual births of SCD infants in the world reaching about 11,000 births per year [3]. The prevalence of SCD among Tanzanians is about 13% [3]. SCD is caused by mutation in the β-chain of hemoglobin (Hb) where the amino acid glutamate is replaced with valine which causes polymerization of red blood cells (RBC) in low oxygen tension, eventually leading to hemolysis and occlusion of blood vessels [4]. These changes clinically present with severe musculoskeletal pain and anemia which may end up with end organ damage or death [5].

Currently, hydroxyurea (HU) is the drug of choice for management of SCD globally. One of the beneficial effects of HU is the induction of the synthesis of fetal hemoglobin (HbF) which circumvents the polymerization caused by HbS hence, evading hemolysis and vaso-occlusion [6]. The pediatric dose of this medication is 20

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mg/kg but the available formulation is a solid dosage form (a capsule) most commonly at a strength of 500 mg [7]. This is too large to match with the pediatric dose, considering body weight of many children under the age of five years. Furthermore, compounding of extemporaneous preparations as a remedy for the aforementioned challenge faces drawbacks such as dose inaccuracy due to wastage of powder during capsule opening, drug degradation when hot water is used for compounding and poor palatability [8]. Poor palatability may have a psychological impact to the children thus, affecting adherence to treatment [9]. The rate and extent to which a drug undergoes hydrolysis is very important in selecting a suitable dosage form for a particular active pharmaceutical ingredient (API). Amide-containing drugs such as HU are very prone to hydrolysis hence dry syrups for reconstitution are the preferred dosage form when a liquid formulation is required [10]. HU undergoes hydrolysis to form hydroxylamine which is pharmacologically inactive [11]. Limited information is available on the rate and extent of hydrolysis of HU in aqueous solution [12].

Dry syrup refers to granules or powder blends which need to be reconstituted at the time of administration when intended to be taken orally [13]. Even so, for highly hygroscopic and hydrolysable API such as HU, a powder blend is more suitable than granules [14]. Dry syrup maintains physicochemical stability for 7-14 days either under refrigeration or at room temperature upon reconstitution [15]. Dry syrups have a number of advantages over conventional syrups such as ease of storage, transportation and enhanced stability as the API is exposed to aqueous environment only at the time of administration [9]. Safety of the pediatric population and compatibility with the API are prerequisites for selection of excipients to be used during the development of HU formulation. In a recent study, inclusion of coloring and flavoring agents raised a number of concerns on the carcinogenic potential of those excipients. Therefore, often the smaller the number of excipients, the safer the HU formulation [16]. So far, there is no HU dry syrup in the market. This study reports the first dry syrup formulation which is expected to solve some challenges in the management of SCD in the pediatric population thus improving treatment outcomes and quality of life.

MATERIALS AND METHODS

Study design

This was an experimental study. It was carried out at the Chemistry Institute of the University of Bergen-Norway, Research and Development Laboratory and the Bioanalytical Laboratory of the School of Pharmacy, Muhimbili University of Health and Allied Sciences (MUHAS), Dar es Salaam, Tanzania.

Materials

Analytical-grade reagents and chemicals were purchased from Sigma-Aldrich Life Science Company (Bergen, Norway). These included HU powder, uracil powder, D-mannitol powder, sodium citrate powder, methanol 98% v/v, acetonitrile, potassium bromide, xanthan gum and aerosil 200.

Determination of hydrolysis kinetics of aqueous solution of hydroxyurea

This study was performed after minor modification of the HPLC method described by Pluscec *et al* in 1986 [18]. A 2 mg/ml stock solution of HU was prepared and divided into three aliquots which were stored at 4 °C, 22-25 °C and 50 °C for 90 days**.** Each aliquot contained 0.25 mg/ml uracil solution as internal standard. Changes in concentration against time for all three aliquots were recorded daily for the entire study period.

Chemical compatibility

A previous described method by Venkateswarlu *et al* 2016, with minor modification using Fourier transform infrared spectrophotometer (FTIR), was used to assess chemical compatibility between Hydroxyurea and excipients in a 1:10 mixture [17]. The FTIR spectra were acquired and analyzed on the 1st and 28th day [17].

Formulation of the dry syrup

Six trial formulations of dry syrups, namely the control and F1a to F1e, were prepared as powder blends by varying excipient ratios while keeping the quantity of HU constant in all formulations. The finished pharmaceutical product (FPP) was designed to carry 2 gm of HU in a bottle to be reconstituted to 100 ml, such that each 5 ml of the reconstituted pharmaceutical product would contain 100 mg of HU. This formulation strength would allow for individualization of the dosage based on child's body weight. Excipients used in the preparation of these formulations were xanthan, aerosil 200, sodium citrate, sodium benzoate and mannitol, all within pharmaceutically acceptable ranges. The master formula has not been disclosed here due to patent restrictions.

Evaluation of the formulated dry syrups

Powder flowability

Angle of repose (AR), bulk density (BD), tapped density (TD), compressibility index (CI) and Hausner's ratio (HR) were used to assess powder flowability for all powder blend formulations as described by Shah *et al* and in the USP protocol document number 1174 [14].

Assay of hydroxyurea dry syrup formulations

A quantity of powdered drug equivalent to 100 mg of API was dissolved in 10 ml distilled water in a volumetric flask. 5 ml of this solution was suitably diluted to make an ideal concentration of HU of 0.25 mg/ml. These solutions were quantified by HPLC method as described by Pluscec and Yuan [18].

Instrumentation

The HPLC system used was an Agilent version 1260 infinity II with both UV and diode array detectors (Agilent Technologies Inc, Santa Clara, CA, USA). System management and data acquisition was performed by the Agilent Chemstation software. HPLC was performed under reverse phase in isocratic mode at flow rate of 1 ml/min. The UV detectors were set at 214 nm and 254 nm.

Chromatographic conditions

A 3.5 µm particle size, 150×3.0 mm column (Agilent Technologies Inc, Santa Clara, CA, USA) was used for the analysis. The mobile phase consisted of methanol-water (30:60).

Preparation of stock and standard solutions

Stock solutions containing 2 mg/ml of HU and 0.25 mg/ml uracil solution as internal standard were prepared using distilled water and stored at 4 °C. The standard solutions were prepared by diluting the stock solution to obtain six standard calibration curves in a range of 0.03125 mg/ml to 1 mg/ml.

Dissolution of hydroxyurea dry syrup formulations

Drug dissolution profile was studied as per the USP monograph [19] using a type 2 apparatus paddle operated at 50 rpm. The dissolution medium was 900 ml of purified water equilibrated at 37±0.5 °C. Powder blend equivalent to 100 mg of API was placed in dissolution vessels 1 to 6, vessel number 7 was the blank whilst vessel number 8 had 100 mg of reference standard of API as comparator [19]. HU was quantified from the solutions by HPLC using the same conditions as those used in the assay. The percentage drug released against time was recorded every 5 minutes starting from the 10^{th} to the 35^{th} minute [19].

Moisture content of hydroxyurea dry syrup formulations

Moisture content was determined by loss on drying method as described in the USP monograph document no 731 [20]. To this end, 2 g of each powdered formulation was heated at 60 °C for 3 hours, cooled and the change in weight recorded. This procedure was repeated several times until no change in weight was observed.

Rheology of the hydroxyurea reconstituted dry syrups

Kinexus rotation rheometer with 60 mm disc diameter was set to produce a shear at 100 rpm. Shearing stress (F) was applied on 1 ml of reconstituted dry syrup from each formulation placed on the disc and the generated rate of shear (G) was recorded in order to determine nature of rheograms.

pH of reconstituted hydroxyurea dry syrups

The measurement of pH was done as per USP document no 791 and a method previously described by Akre *et al* [9] with minor modification. The reconstituted syrups were stored under refrigeration at 4 °C and at room temperature (22-25 °C) with the change in pH for a period of 14 days recorded.

Ethical consideration

Ethical clearance for performing this study was granted by MUHAS institutional review board, reference no. MUHAS-REC-04-2020-218.

RESULTS

Chemical compatibility

All excipients were chemically compatible to HU as evidenced by retaining similar FTIR spectra wave numbers on day 28 (Table 1).

Hydrolysis kinetic of aqueous hydroxyurea solution

No hydrolysis of HU was observed on the stock solutions when stored at room temperature (22-25 °C) and at 4 °C in the refrigerator (Table 2). However, a stock solution stored at 50 °C for 90 days demonstrated a linear decrease in concentration when plotted on a Cartesian graph suggesting zero-order kinetics (Figure 1).

Flowability of powder blends

All formulated powder blends demonstrated good to excellent flowability (Table 3).

Assay

Of the six formulations developed and subjected to the assay test, all complied with USP requirements (90-110%) except formulation F1c which had a higher concentration. The concentration of HU in the assayed formulations were as follows: - control (99.6 ±0.25%), F1a (104.8±0.26%), F1b (105±0.26%), F1c (113.2±0.26%), F1d (100±0.25%), F1e (106±0.27%).

Dissolution test

All the formulated powder blends subjected to the dissolution test complied with the USP requirements by releasing more than 85% of HU within the first 30 minutes (Figure 2).

Table 1: **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 1st day	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 1st day	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 1st day	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 1st day	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 1st day	-	-	-	-	-	-	-	-	-	(1060, 799),
HU+Aerosil 28th day	-	-	-	-	-			-	-	(1070,796), no reaction
HU+Sucralose 1st day	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	
HU+ALL EXCIPIENTS 1st day	3150-3320			1610	1430-1510	1650		1370	1220	No reaction
HU +ALL EXCIPIENTS 28th day	3160-3320			1610	1430-1500	1650	1020	1370	1220	

Table 2: Stability of hydroxyurea aqueous solutions at different conditions of temperature

Storage temperature	Hydroxyurea Concentration (mg/ml) on day 1 to 54							
	1	**7**	**9**	**13**	**25**	**30**	**39**	**54**
Solution at 4 °C	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Solution at 22-25 °C	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Solution at 50 °C	2.00	1.98	1.98	1.89	1.54	1.09	0.66	0.24

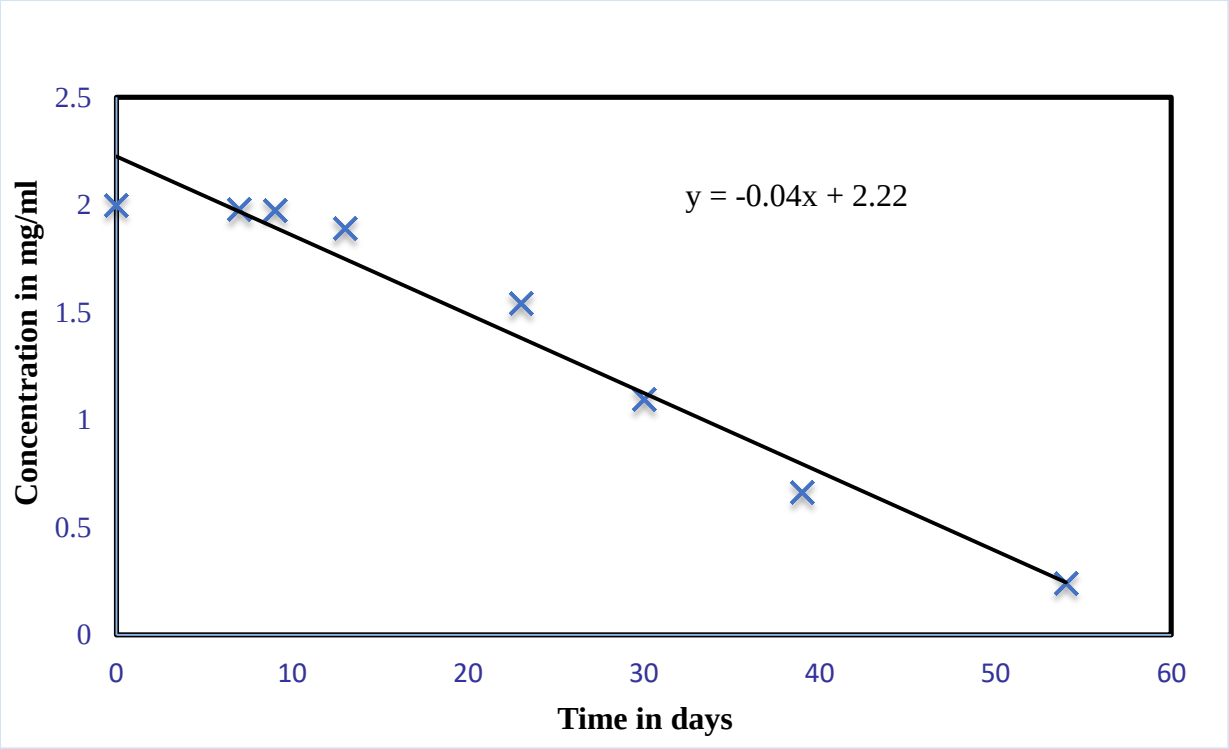


Figure 1: Zero order hydrolysis exhibited by hydroxyurea aqueous solution stored at 50°C

Table 3: Flowability parameters of various powder blends

Formulations	Flowability				
	Angle of repose	Bulky density	Tapped density	Compressibility index	Hausner's ration
Control	25.240	0.823	0.980	16.050	0.840
F1a	24.700	0.823	0.952	13.580	0.980
F1b	24.420	0.830	0.935	11.200	0.880
F1c	26.220	0.826	0.935	11.570	0.880
F1d	24.907	0.800	0.926	13.600	0.860
F1e	25.333	0.704	0.800	11.970	0.880
Remarks	Excellent in all except F1c (good)	Less air accumulation	Less air accumulation	Excellent in all formulations	Excellent in all formulations

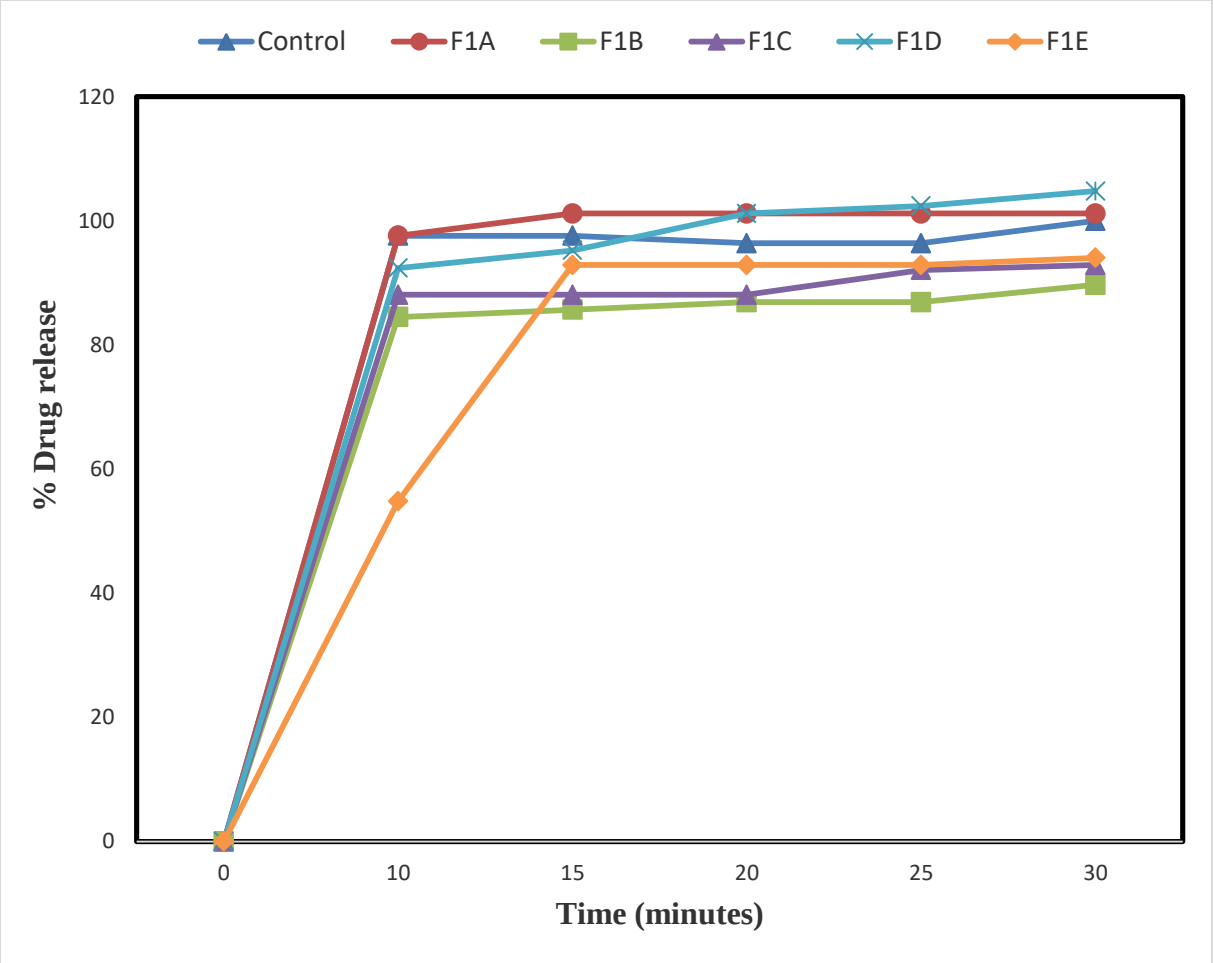


Figure 2*:* Dissolution profile for all dry syrup formulations

Moisture content

The moisture content of all the developed formulations was within the acceptable limit of not more than 5%. The determined moisture content was as follows: control (0.16%), F1a (0.05%), F1b (0.06%), F1c (0.04%), F1d (0.01%) and F1e (0.36%).

Rheology of reconstituted dry syrups

All reconstituted syrups demonstrated pseudoplastic flow, which is the most desired behavior for pharmaceutical products developed as solutions and suspensions (Figure 3).

pH of the reconstituted dry syrup

All the reconstituted syrups stored at 4 °C in the refrigerator and at room temperature (22-25 °C) for 14 days passed the pH test as per USP requirements (pH 5-8.). The syrups maintained a pH range from 7.3 to 7.6, indicating chemical stability of the reconstituted syrups.

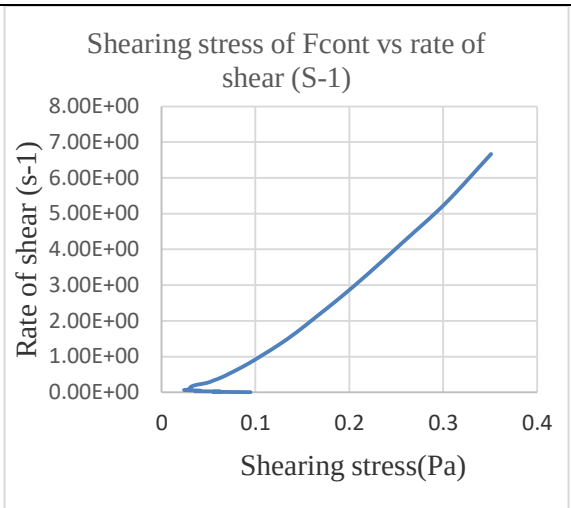
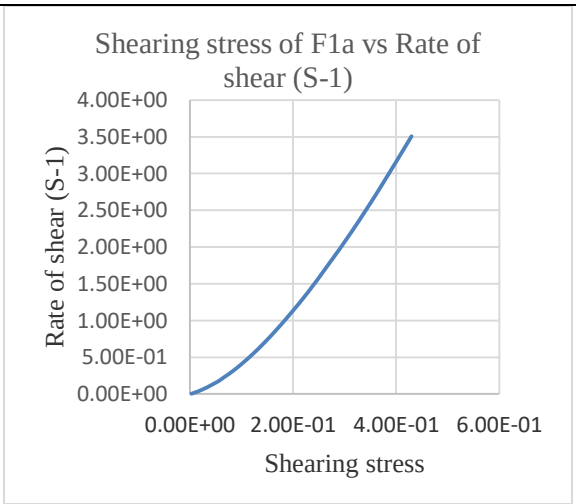
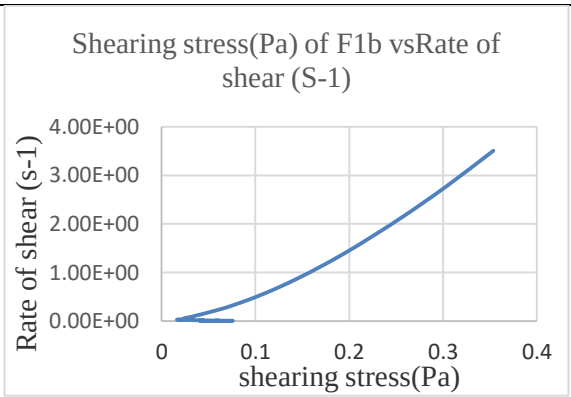
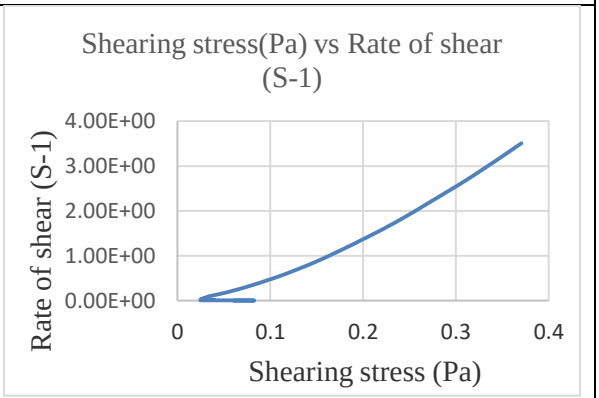
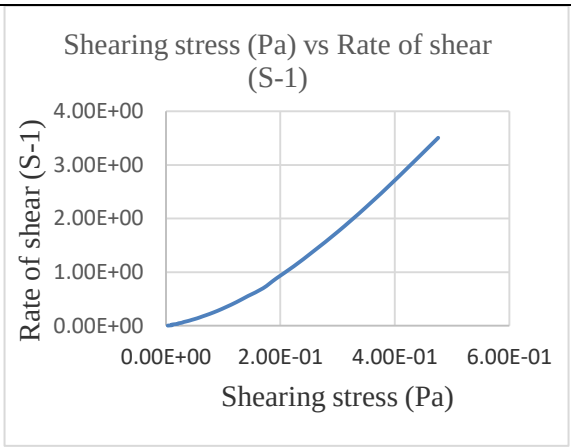
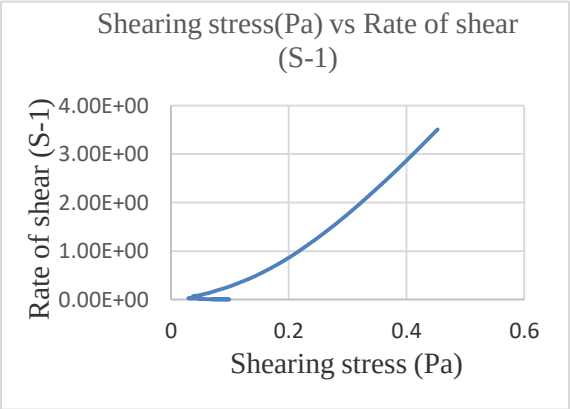
S/N	Fcontrol	F1a
1		
2	F1b	F1c
		
3	F1d	F1e
		

Figure 3: Pseudoplastic flow of all HU dry syrup formulations

DISCUSSION

The management of pediatric patients with SCD is currently hampered by the lack of appropriate formulations of HU. This study reports the first dry syrup formulations of HU which demonstrated acceptable specifications as per USP requirements [1]. These formulations hold potential for future development and use in pediatric population with SCD.

In this study, HU solution was found to be stable for 90 days when stored at (4 °C in the refrigerator and 22-25 °C at room temperature. These findings corroborate results from a previous study by Heeney *et al*. in which an extemporaneous preparation of HU capsule was found to be stable for 90 days at when stored at 4 °C in the refrigerator (8). These findings give insights to manufacturers on the possibility of large-scale production of HU dry syrups.

The values of flowability parameters namely AR, BD, TD, CI and HR complied with the USP-flowability specifications [21]. 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 [14].

Of the developed formulations of HU all with an exception of F1c conformed to assay test specifications as per USP specifications which reflects uniform distribution of API during mixing [22]. Lower or higher assay values may indicate that the API was not uniformly distributed during mixing of powder blend or granules for dry syrup. The use of finished pharmaceutical product (FPP) with higher API content has a potential to cause toxicity and death in some cases 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 [22]. This implies that the FPP upon administration will release sufficient medication at the absorption site [23]. Poor dissolution of the medicine is associated with poor therapeutic outcomes.

Moisture content of the powder blends or granules determines the chemical stability, powder flowability, probability of powder caking as well as the chance of microbial growth in the formulation [24]. In the current study, MC for all the developed formulations were less than 1% which is within the required limit of pharmaceutical powders of less than 5% [25]. For formulations with hygroscopic API such as HU, the lower the MC value the more stable the formulation.

The recommended pH for dry syrup upon reconstitution is between 5 and 8 for 14 days [8]. Findings from this study were in line with this recommendation and with a previous study conducted by Harshada *et al.* [9]. In the current study, no significant change in the pH was observed in all the developed dry syrups upon reconstitution and storage for 14 days. This indicates absence of hydrolysis or degradation of API by any mechanism [8,11].

Viscosity is an important parameter as far as a liquid dosage form is concerned as it affects drug release from the dosage form into gastrointestinal fluid [23]. High viscosity creates a barrier through which a drug must permeate to reach the biological membrane. The pseudoplastic flow exhibited by these syrups is a desired viscosity property for pharmaceutical suspensions or solutions [23]. These findings are in line with the study conducted by Bila *et al.* where xanthan polymer exhibited pseudoplastic flow in a number of formulations [23]. This shear thinning property allows a decrease in viscosity upon shaking so that the drug can be easily released upon administration.

The particle size analysis of the powdered excipients and API was not studied due to absence of an appropriate filter. However, this was mitigated by procuring finely powdered ingredients of laboratory scale.

CONCLUSION

HU dry syrup was successfully developed and evaluated. Formulation F1d was the most ideal in all aspects studied and thus shows potential for further optimization to a product that would address current challenges in the management of SCD in pediatrics. Real-time and accelerated stability studies should be performed so as to establish the shelf life of the formulation.

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