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Extemporaneous Furosemide Suspensions for Pediatrics Use Prepared from Commercially Available Tablets

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ABSTRACT

Objective: Three furosemide aqueous suspensions for paediatric oral usage (2 mg/ml) were investigated to determinate its physicochemical stability under different storage conditions. Method: Formulations using sugar syrup and sugar-free vehicles were stored at 4 and 25°C and the furosemide content was determined by spectrophotometer. Each sample was analyzed in triplicate at different time points (0, 7, 14, 28, 56, 72 and 90 days). Results: Liquid suspensions were successfully formulated from commercially available tablets. In both cases, samples showed suitable physical stability. Furosemide was chemically stable in aqueous suspension during the 90 days of the study at the two storage temperatures. Conclusions: All the tried oral liquid formulations can be conserved at 4 and 25°C at least 56, 72 and 90 day period.

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1:0 INTRODUCTION

The lack of commercially available oral liquid dosage forms is an ongoing problem in many practice settings. A pharmacist is often challenged to provide an extemporaneous oral liquid for Pediatric patients; Patients who are unable to swallow solid dosage forms such as tablets or capsules; Patients who must receive medications via nasogastric or gastrostomy tubes; Patients who require non-standard doses that are more easily and accurately measured by using a liquid formulation [1].

For oral administration, furosemide is available in UAE as tablets 40mg. The oral liquid dosage form is not available, although it is manufactured in other countries. Pediatric demands for this product in clinical settings necessitate pharmacist involvement in retaining compounding skills to extemporaneously prepare such products in stable oral liquid forms[2]. The lack of paediatric liquid dosage forms represents a challenge for hospital and community pharmacists. Usually, children require smaller doses than adults which are adjusted by body weight. Thereby solid dosage forms must be fractionated in order to fit paediatric dosages. This practice represents a concern issue because correct dosing must be ensured[13]. Preparing extemporaneous liquid formulations using tablets is one of the most common practices employed to adjust doses for paediatric patients. Moreover, children under 7 years old are unable to swallow capsules or tablets. Liquid formulations which are flavored aqueous solutions, syrups, or suspensions, are administered directly into the child's mouth by drop, spoon, or oral dispenser or incorporated into the child's food14. General dose volumes are < 5 ml for children under 5 years and < 10 ml for those of 5 years and over15.

Among paediatric pharmacy practice, traditional compounding techniques have become a useful tool in order to develop not commercially available liquid dosage forms[17].

In this context, liquid formulations facilitate oral administration and enhance children treatment adherence. An appropriate design of a liquid dosage form requires not only a comprehensive analysis of chemical and physical considerations of drugs and pharmaceutical excipients but also evaluation of drug stability in the mid-long term as well as drug effectiveness, tolerance and formulation safety[17].

Studies [2, 7, 9, 11-14] have identified that the preparation of liquid formulations for paediatric patients is both a daily experience and challenge for the pharmacist and paediatric health care provider. Appropriate formulations for administration to children exist for only a minority of commercially available drugs and the need for extemporaneously compounded formulations is escalating due to the release of many new drugs formulated for adults but with expected use in children [7, 9, 11]. Children require titratable individualised doses in milligrams per kilogram of body weight and most children under six years of age cannot swallow tablets [15, 16]. A survey [14] into the informational needs of hospital compounding pharmacists providing pharmaceutical care to paediatric patients at 57 sites in the USA and Canada listed 76 extemporaneously prepared drug formulations as having adequate stability data, 109 formulations for which improved stability data were requested, and an additional 103 drug formulations prescribed by paediatricians that had no compounding or stability information available. There are many reasons for the lack of commercially available paediatric formulations. The overall size of the paediatric market is much smaller than for adults, especially for common diseases such as hypertension. The industry is thus reluctant to commit resources to seek labelling for infants and children (unless a disease occurs exclusively or frequently in the paediatric population) since the formulation has to have been adequately studied in paediatric patients. Therefore, additional costs, limited financial returns, delay in marketing for adults, and perceived greater legal liability and regulatory requirements are impediments to developing and marketing a paediatric drug formulation [7, 17]. Tablets are often cut into smaller segments (halves or quarters) in the pharmacy or on the ward to obtain appropriately sized dosage units for children, however, a major concern is that segments from tablets cannot be cut with great accuracy of dose [12, 19-21].

McDevitt et al [20] conducted an extensive analysis on the ability to split a 25-mg hydrochlorothiazide tablet accurately by 94 volunteers. Of 1752 manually split tablet portions, 41.3% deviated from ideal weight by more than 10% and 12.4% deviated by more than 20%. gender, age, education, and tablet-splitting experience were consistently found not to be predictive of accuracy. Most subjects (96.8%) stated a preference for commercially produced, lower-dose tablets, and 77.2% were willing to pay more for them. The issue of cost containment in the treatment of hypertension has seen many physicians prescribing larger dosages of drugs and then instructing patients to split the tablets to receive the correct dose, and some health

maintenance organisations are providing tablet splitters to patients while dispensing larger than prescribed doses [20]. Another practice seen in paediatric care is to use injectable solutions for oral administration [13, 26].

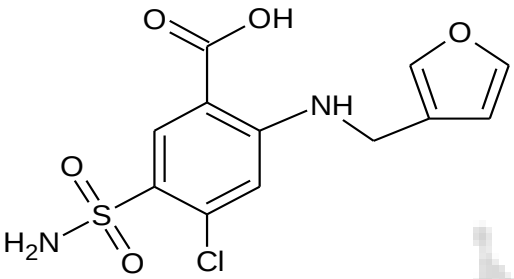
When a drug is formulated for paediatric use, several factors unique to paediatrics must be considered such as the immaturity of the intestinal tract and the subsequent influence on gastrointestinal absorption, seriously ill neonates are often fluid restricted, limiting the volume of medications that can be received. Additives, including preservatives and sugar must be chosen carefully. Patients who are fructose intolerant have had significant adverse effects from sorbitol and there is a link between chronic uses of sugar-sweetened medication and dental caries [11]. Formulations may also contain preservatives; an excipients considered to be largely inert in adults, however, may lead to life-threatening toxicity in paediatrics when multiple doses of medications with the same preservative are employed. This is particularly the case with benzyl alcohol and benzoic acid [11]. The physical, chemical, microbial and therapeutic stability of the above paediatric extemporaneous preparations may not have been undertaken at all. This coupled with the increased potential for calculation or dispensing errors may prove the practice of modifying commercially available products to be extremely unsafe. Although information [29-31] is available detailing extemporaneous formulations for parenteral and oral use, however, only some of the formulations have documented stability data.

FUROSEMIDE

Pharmacology: (FSM) is a potent diuretic widely used in the treatment of edema associated with heart and renal disorders [1]. Furosemide primarily inhibits sodium and chloride reabsorption in the thick ascending limb of the loop of Henle [2] promoting increased elimination of potassium, magnesium, calcium and, to a lesser extent, bicarbonates. Furosemide is a loop diuretic used in the treatment of congestive heart failure and edema[1]. Like other loop diuretics, Furosemide acts by blocking the Na^+-K^+-$2Cl^-$ symporter in the thick ascending limb of the loop of Henle, decreasing the sodium reabsorption from the tubular fluid resulting in increased water secretion into the tubule and hence reducing the blood pressure[2]. Furosemide is listed as an essential drug by the World Health Organization (WHO, 2009). Although newer drugs are available,

furosemide is routinely prescribed in both developing and developed countries (Kaufman et al., 2002) due to their low cost and efficacy.

Drug profile



IUPAC Name: 4-chloro-N-furfuryl-5-sulphamoylanthranilic acid.

Molecular formula: $C_{12}H_{11}ClN_2O_5S$**.**

Molecular weight: 330.75g/mol.

Description: It is a White or slightly yellow Crystalline powder.

Solubility: Freely soluble in acetone, soluble in methanol, sparingly soluble in ethanol & practically insoluble in water.

Category: Diuretic drug.

2:0 MATERIALS AND METHODS

Working standard of Furosemide as a gift from Julphar Co (RAS, UAE.), furosemide 40 mg tablets (Hoechst Marion Roussel) and date of production is 23-2012, sodium carboxymethylcellulose, methyl and propyl paraben, Potassium carbonate, potassium dihydrogen orthophosphate sodium hydroxide (Gulf Pharmaceuticals, RAK, UAE),); Tween 20 and 80, Ethanol 95%, Sucrose, Citric acid monohydrate (M/S Merck, Germany), Sorbitol, glycerol, (source: Al Khaleej, Dubai) Raspberry syrup, Caramel color 3M, Aspartam (M/S Cerestar Ltd, UK). All materials are of analytical grade and conform to specifications of pharmacopoeia (BP, USP). (Hopkin and Williams LTD, England).

2.1: Preparation of furosemide 2mg/ml suspensions:

In the present study, we prepared three FSM 2mg/ml (equivalent to 0.2% w/v of FSM) suspensions from commercial tablets 40 mg, in three compounded vehicles denominated M1, M2 (sugar based vehicles) and M3 (sugar-free based vehicle). Detailed methods of preparation followed by the composition of suspensions are shown below and in Table 1.

2.2: Preparation of sugar syrup (66% w/w):

100 gm sugar syrup was prepared. First 34 gm of purified water was heated to $70^0C \pm 1^0C$ in a beaker in water-bath. Then 66 gm of sucrose was dissolved in the hot water in the beaker with stirring. After that, the temperature was raised to $90^0 \pm 1^0C$. At this temp, the sucrose solution was heated for 45 minutes. Then syrup was cooled to 40^0C. Finally, weight of sugar syrup was adjusted to 100 gm with purified water and stir for 2 minutes. Thus, sugar syrup was prepared and considered as M1.

2.3: Preparation of partially inverted sugar syrup (66% w/w):

100 gm partially inverted sugar syrup was prepared. First 34 gm of purified water was heated to $70^0C \pm 1^0C$ in syrup a beaker in water-bath. 0.022 gm of citric acid monohydrate was dissolved in 1 gm of purified water and added to the beaker. Then, 66 gm of sucrose was dissolved in the hot water with stirring. After that, the temperature was raised to 90 ± 1^0C. At this temp, the sucrose solution was heated for 45 minutes. Then the syrup (inverted) was cooled to 40^0C. Finally, weight of sugar syrup (inverted) was adjusted to 100 with purified water. Thus partially inverted sugar syrup was prepared and considered as M2.

2.4: Preparation of 2% SMC suspending vehicle preparation:[8]

1. Heat 20 mL of distilled water to boiling.
3. Wet 2g of SMC powder and add it to the mixture.
4. Allow the mixture to stand for 15 minutes, and then remove from heat.
5. Add to 70 mL of cold distilled water while mixing well with a magnetic stirrer.
6. Keep mixing until a clear, homogeneous solution result.
7. Mixture of banana flavor, sweetener was added, gradually with stirring.

8. Finally, the suspension volume was brought to 100 ml with distilled water.

2.5: Preparation of FSM 2mg/ml suspension:

Five tablets of furosemide 40mg (equivalent to 0.2% w/v of FSM) were finely ground to a fine powder using pestle and mortar was used to crush the tablets. A small amount of syrup, or invert syrup or prepared 2% methyl cellulose was added levigated to a thick, smooth paste. Then the remainder of the required vehicle was added by geometric dilution. Other added were dissolved and then added to previous mixture. Bring the suspension to a final volume using the required vehicle. Mix briefly with a mortar and pestle until a uniform suspension is formed. Transfer to 60 ml amber bottle and store away from light at two controlled temp. 4^{o}C and 25^{o}C.

Table 1. Composition of liquid vehicles used in the study [% w/w][1]

Pharmaceutical excipients	Functional Category	M1	M2	M3
		Formulation (% W/V)		
Sucrose (g)	gnineteewS	66.0	66.0	
Glycerol (ml)	Humectant	4.0	4.0	
Sorbitol 70% Sol. (ml)	Humectant and sweetener	5.0	5.0	
Methyl Cellulose (g)	Suspending agent			2% Sodium Methylcellulose
Tween 20 (ml)	Solubilizing	1	1	
Citric acid (g)	Sucrose hydrolysis catalyst		1.0	
Potassium Sorbate (g)	evitavreserp laiborcimitnA	0.08	0.08	
Aspartame (g)	Sweetening	0.3	0.3	0.3
Banana caramel syrup (ml)	Flavor &colour	10	10	10
Water (ml)		to 100.0	to 100.0	to 100.0
pH		4.5	4.7	6.0
Viscosity [mPas]		72	62	100

M1: Sugar syrup vehicle, M2: Partially inverted sugar vehicle, M3: Sugar-free vehicle

2.6: Determination of standard curve of furosemide in phosphate buffer 5.8

Stock solution of analytical grade furosemide was prepared by accurately weighing furosemide 10mg, dissolving 100 ml phosphate buffer 6.8, to obtain a series of solutions, (5, 10, 15, 20 and 25) µg/mL respectively. The absorbance of each solution was measured at 276nm.

The standard curve (n=3) was constructed by plotting the absorbance at of furosemide solution against its corresponding concentration and was used for calculating the drug concentration of the sample. The standard curve was linear (r^2>0.999) over the working range of concentrations. Furosemide concentrations were assayed in triplicate.

Solution No.	Conc. (µg/mL)	Absorbance at 276 nm
1	5	0.231
2	10	0.429
3	15	0.624
4	20	0.779
5	25	0.967

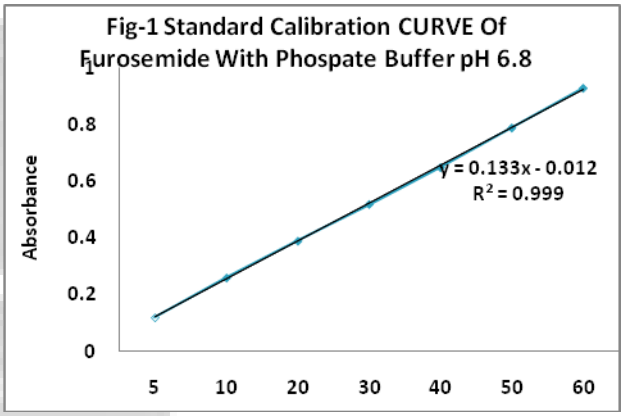


Figure 1. Concentration of furosemide standard and their absorbencies

A standard curve was constructed from the standard solution. The values of absorbance of standard solutions were plotted vs. the values of their corresponding values concentrations to make calibration curve. This standard curve was used to determine the drug content or concentration (assay) of furosemide in the suspensions and the drug release during dissolution studies.

2.7: Preparation of Phosphate buffer pH 6.8:

Phosphate buffer pH 8 was prepared by mixing 50ml of a solution of 0.2 M potassium dihydrogen orthophosphate with 46.8ml of 0.2M sodium hyproxide then diluted to 200ml with water.

3.0: EXPERIMENTS

3.1: Determination of Inversion Measurement of pH determination

The apparent pH was determined at 0, 28, 56 and 91 days. Examination was conducted with a digital pH-meter IQ 140 (IQ Scientific Instruments, CA, USA). Each measurement was done in triplicate and then the results were averaged.

3.2: Viscosity determination apparent viscosity:

Viscosity, which is the inverse of fluidity, could vary after storage under different conditions. Thereby, samples (150 ml) viscosity was tested, with Brookfield DVII+ Pro viscometer which read shear stress versus shear rate and the results were averaged. Measurement was performed on day 0, 28, 56,72 and 90. Viscosity was measured three times in units (CP) and the results were averaged. Rheogram was obtained for the selected formula at 37^{o}C.

3.3: Resuspendability of suspension

The resuspendability is a quantitative test to evaluate the ease of redispersion of a suspension after a long period of standing[3].

The efforts required to convert the sedimented system to homogenous suspension upon shaking the cylinder manually, were rated with a ranks as follows: resuspendable, resuspendable with difficulty or not resuspendable[4]

3.4: Sedimentation volume

A suitable parameter, for assessing the physical stability of a suspension, is the sedimentation volume which is defined as:

$$V_s = V_f / V_o$$

Where Vs is the volume ratio, V_f and V_o are the final settled volume and the original suspension volume, respectively. In the present study, samples (50 ml) were hand shaken for 30 seconds in order to assess the content uniformity and transferred from amber glass vials to a 50 ml graduated cylinder protected from light and sealed with a rubber cap. Then, V_s was determined after samples were allowed to stand for 56 days at 4 and 25^{o}C. Assays were done in triplicate and then the results were averaged.

3.5: *In-situ* observation crystal growth in sugar base FSMM1 & FSMM2 formulations

The suspensions were examined for crystal growth observation, in opened petri-dish, bottle neck and cap of sugar syrup containing preparations.

3.6: Opened Petri-dish:

25gm from sugar syrup formulations was kept in opened glass petri-dish without disturbance, for 36 hrs, 2 weeks & 1 month observation for crystal growth of sucrose in the syrup at room temperature were monitored & results were recorded. The vehicle without addition of the drug considered as control.

3.7: Dissolution rate measurement

The dissolution medium was 900ml of phosphate buffer 6.8. The temperature of the study was 37^{0}C and the rotating velocity was 100 rpm min^{-1}. 5 ml of each formula 1, 2 and 3 was transferred to the jar bottom using a syringe. At appropriate intervals samples of 5ml were taken from the jar and analyzed for total content of furosemide by UV-spectrophotometer. Detection was done at 330nm. 5ml of fresh phosphate buffer was added to the jar with each time intervals to keep the volume constant.[5]

3.8: Microbiological stability

The samples were subjected to microbiological evaluation. The criteria is the attributes of non-sterile pharmaceutical products which is set as total microbial count below 200 CFU/ml, total

and absence of *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli and Salmonella spp*.[6] Microbacterial examination was performed by the Department of Microbiology.

Total microbial count tests were performed to determine the microbial contamination of the samples at the same time of physical and chemical testing. Tryptic soy agar was used as the media. Testing was performed in laminar air flow using aseptic techniques. The samples were compared to positive control utilizing *S. aureus, P. aeruginosa and E. coli* as test microorganisms and negative control.[7] Briefly, 5 ml of the sample was diluted with 45 ml of phosphate buffer pH 7.2 and mixed. 1 ml of the mixture was transferred onto a plate containing tryptic soy agar. The mixture was mixed gently, followed by incubation at 35°C for 24-48 h, and the microbial contamination was counted. Each sample was run in duplicate.

3.9: STABILITY STUDY

In order to evaluate FSM chemical stability under different storage conditions, FSM suspensions were storage at 4 and 25^{o}C in amber, glass bottles (60 ml) in the absence of light. Then, aliquots were collected from each container on days 0, 3, 7, 14, 28, 56, 72 and 90. Samples were analysed by spectrophotometer at 276 nm[8]. FSM stability in each liquid formulation was determined by calculating the percentage of the initial FSM concentration remaining at each time point. Stability was defined as the retention of not less than 90% of the initial concentration.

Data analysis:

The initial concentration of furosemide was defined as 100% and sample concentrations were expressed as a percentage of the initial concentration remaining. The stability of the drug was defined as not less than 90% of initial drug concentration remaining in the preparation. The significance of any difference between initial and final pH values was evaluated by a Student's t-test.

4.0: RESULTS AND DISCUSSION

The aim of the present study was to develop an optimal oral liquid FSM in order to enhance physical-chemical stability of the drug and paediatric patient acceptance.

Preliminary studies investigated the development of FSM 2mg/ml solution with a high concentration of co-solvent such as propylene glycol (60% v/v) and sorbitol 70% solution (30% v/v), in order to enhance FSM aqueous solubility. However, FSM extemporaneous solution presented low physical stability and drug remaining in solution only for 7 days. Also, formulations with lower concentration of propylene glycol presented drug precipitation at 48 h. Therefore, an aqueous suspension results in a useful dosage form for administering a water-poorly soluble or aqueous chemical unstable drug. This is the main reason why suspension development appears as an excellent approach for FSM liquid formulation containing a quantity of drug in an acceptable volume[1]. Also, suspensions could mask FSM unpleasant taste improving paediatric formulation acceptance and treatment adherence. Extemporaneous liquids with FSM were prepared using three different compounded media: M1 and M2 were sugar syrups including simple syrup and inverted syrup. Medium M3 was sugar-free 2% sodium methyl cellulose. All compounded suspending media were prepared according to the US monograph vehicle for oral solution, with addition of 1% citric acid with heat in M2 for inversion process. M1 and M2 was sucrose syrup with sorbitol and glycerol (Table 1).

The vehicles M1 and M2 contain potassium sorbate as a preservative. Neither detectable changes in color or odor and taste, nor visual microbial growth were observed in any formulation prepared with FSM, stored at 25^0C or 4^0C, in spite of the fact that possibility of fungal growth was reported if a drug was compounded by media without preservatives[3].

Although we did not observe any microbial growth in a preservative free M3 medium, however, according to the USP guidelines only 14 days expiry date can be proposed when a compounded liquid does not contain preservatives. Benzoates and parabens were excluded from all formulations because they are ineffective as preservative in alkaline media which FSM freely soluble in it.

The presence of glycerin and sorbitol in media M1 and M2 contributes to solvent effect, assists in the dissolution of the solute, adds to the viscosity and enhance the stability of the preparation. However, the presence of excessive amount of these materials also. However, sorbitol, glycerin, and propylene glycol according to literature they have been reported to cause side effects in children [4,5], particularly the neonatal and infant age groups.

To minimize the amount of these solvents in these preparations, simple suspending vehicles can be used such as simple syrup, invert sugar syrup and sodium methyl cellulose syrup as in compounding vehicle M1, M2 and M3. The sodium methylcellulose, a derived gum function as viscosity builder agent. [6] Recently, invert syrup which has been used mostly in food and candy, have been employed especially after the dental problems of children teeth decaying is increasing.

All formulations have nearly similar taste but different in intensity. However, the formulation in M2 inverted sugar is better, because the effectiveness of banana flavor in masking the taste of FSM is enhanced by presence of weak acid (citric acid)[7].

4.1: Physical stability

There was no detectable change in physical characteristics such as color, odor, and no visible. In all cases, suspensions were easily re-dispersed after simple handshaking (30 seconds) microbiological growth in any sample during the three months of storage at two controlled temperatures. In the same table results of Microbiological tests showed that no microbial growth was detected. The total of bacterial, yeast and mould count of all samples was less than 10 colonies per g (cfu/g) of the sample. The suspected colony of *E. coli* was not found in Mac Conkey agar medium of any samples Table 2.

Table 3. Results of physical and microbiological tests (furosemide 2mg/sugar syrup vehicles) at 5 ± 3ºC& RT

Lot Detail	Day 0	Day 7	Day 30	Day 56	Day 72	Day 90
Furosemide 2mg/ml sugar syrup suspension						
Redispersibility	1	1	1	1	1	1
Color	0	0	0	0	0	0
Odor	0	0	0	0	0	0

Analysis bacteria	√	√	√	√	√	√
Crystal growth (open petri-dish)	N	Y	Y	Y	Y	Y
Crystal growth (bottleneck)	N	Y	Y	Y	Y	Y
Furosemide 2mg/ml partially inverted sugar syrup suspension						
Redispersibility	1	1	1	1	1	1
Color	0	0	0	0	0	0
Odor	0	0	0	0	0	0
Analysis bacteria	√	√	√	√	√	√
Crystal growth (open petri-dish)	N	N	N	N	N	N
Crystal growth (bottle neck)	N	N	N	N	N	N
Crystal growth (vertical & inverted)	N	N	N	N	N	N
Furosemide 2mg/ ml 2% SMC syrup suspension						
Redispersibility	1	1	1	1	1	1
Color	0	0	0	0	0	0
odor	0	0	0	0	0	0
Analysis bacteria	√	√	√	√	√	√

Note: Analysis bacteria: Total Count (√)0-200 CFU/ml, (✗): Total Count >200 CFU/ml

- Redispersibility: 1: Shake 1-10 times. 2: Shake 1-20 times.

- Color: -1: Light,(0): do not change color, +1: darker, - is no analysis.

- Crystal growth : (N) No growth, Crystal growth : (y)

To assess the FSM stability in pediatric oral formulations, extemporaneous oral liquid FSM suspensions from commercially available tablets were assayed. These suspensions were prepared with excipients commonly used in paediatric formulations[22] and they were stored at two different temperatures (4 and 25°C) for 90 days. The effect of pH on solubility is critical in the formulation of liquid dosage forms. The solubility of FSM (PKa=3.9) is often pH dependent.

Furthermore, pH control is at least as important to fully control the crystalline habit and the stat of agglomeration to ensure quality, efficacy and safety of the drug.[2] Also, tween 80 is added to increase solubility of FSM. Results showed that pH values did not show any important variation along the assay. See Table 3.

Table 4. pH of FSM suspensions in the three media vehicles stored at 4 and 25°C For two temperatures (n = 3)

noitalumroF	**Temperature (°C)**	Hp			
		day 0	**day 28**	**day 56**	**day 91**
FSMM1	4	6.68 ± 0.03	6.65 ± 0.05	6.76 ± 0.08	6.68 ± 0.03
	25	6.68 ± 0.03	6.65 ± 0.04	6.69 ± 0.07	6.49 ± 0.04
FSMM2	4	6.85 ± 0.04	6.85 ± 0.06	6.94 ± 0.100	6.85 ± 0.04
	25	6.85 ± 0.04	6.83 ± 0.07	6.95 ± 0.10	6.64 ± 0.100
FSMM3	4	6.94 ± 0.10	6.94 ± 0.10	6. 76± 0.10	6.65 ± 0.10
	25	6.95 ± 0.10	6.95 ± 0.10	6.75 ± 0.10	6.68 ± 0.03

Oral liquid dosage can be dispensed from a variety of containers, such as plastic and glass bottles. However, they generally dispense the dose by pouring from these bottles.

Caps are placed on these bottles in order to protect the product from drying out due to evaporation of water and crystallization of solid material in the liquid during use by the consumer or left undisturbed for a long period of time, resulting in cap lock problem.

Cap lock is the cementing of the cap on the bottle when liquid unintentionally gets onto the outfits, residues of sugar between the neck and the bottle or tendency of some consumers not to replace the cap between uses. In order to prevent cap lock, humectants are added to oral liquid dosage form by preserving moisture and prevent crystallization. However, to minimize the excessive use reported side effects, inverted sugar syrup was proposed. Invert syrup has proved to be very popular with consumers. Undoubtedly, this is due at least in part to their appealing translucent appearance and surprisingly pleasant taste. Results for crystal growth crystallization is shown in Table 4 and Figure 2.

Figure 2: Results for crystal growth crystallization

However, the Vs increased between 0 and 90 days at both storage temperatures. See Table 4. Notice that formulations stored at 4°C showed lower Vs values than their counterparts stored at 25°C for 28 and 56 days. [17,18]

Table 5. Vs of FSM suspensions (FSMM1, FSMM2 & FSMM3) stored at 4 and 25°C. For two temperatures (n = 3)

noitalumroF	Temperature (°C)	Vs		
		day 28	day 56	90 days
FSMM1	4	0.047	0.061	0.070
	25	0.082	0.079	0.091
FSMM2	4	0.025	0.043	0.053
	25	0.035	0.043	0.052
FSMM3	4	0.057	0.067	0.077
	25	0.082	0.091	0.099

The results show that the sediment volume increases in the following order: FSMM3>FSMM1>FSMM2. The sedimentation volume depends on the types and concentrations of suspending, wetting and viscosity enhancing agents used.

As a result, a high sediment volume observed in formula FSMM3 could be a result of the increase in the viscosity of the dispersion in alkaline media which may be caused by the expansion resulting from electrostatic repulsion between negatively charged carboxylate group. ([17, 18])

The presence of glycerol, sorbitol and Caramel-Banana syrup adds to the viscosity of suspension. It is effective because of its high viscosity and enhance the palatability by coating the tongue and thus it tends to inhibit diffusion of the furosemide to the taste buds.

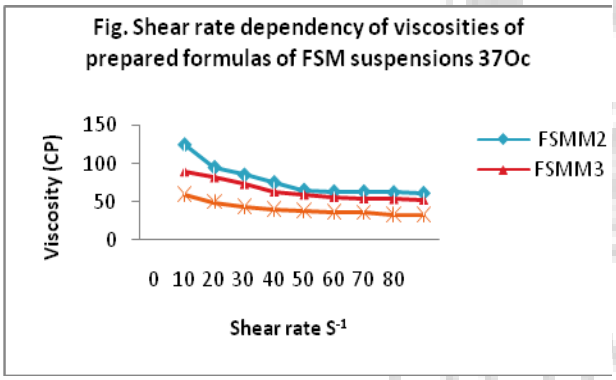
The rheogram of formula FSMM3 has a high viscosity at low shear stress while at higher shearing stress, it has low viscosity due to the flow behavior which is typical pseudoplastic and thixotropic characteristics.(18)

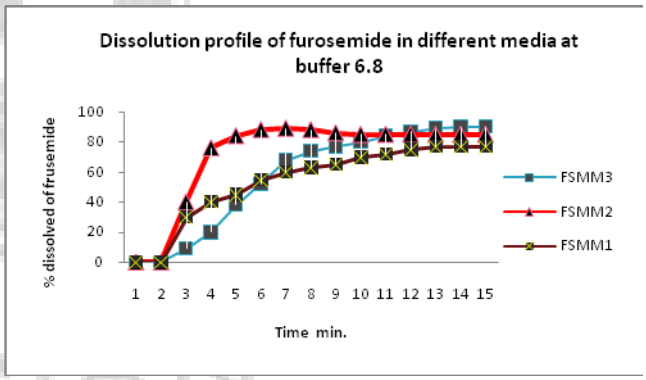
Figure 3 shows the rheogram was obtained for the selected formula FSMM1, FSMM2 and FSMM3, at 37^0C with Brookfield DVII+ Pro viscometer which read shear stress versus shear rate. Formulation FSMM3 showed higher viscosity values than formulation FSMM1 and FSMM2.

Formulation A showed an increase in viscosity values after 28 and 56 days under refrigerated conditions (4^oC). However, the samples viscosity was reduced when the storage was performed at 25^oC Table 4. A similar trend was followed by formulation B. Nevertheless, the viscosity variation at 4^oC was less marked and values remain almost unchanged.

Table 6. Viscosity (mPas) of FSM suspensions in different media vehicles stored at 4 and 25°C. For two temperatures (n = 3)

noitalumroF	**Temp. (°C)**	Hp			
		day 0	**day 28**	**day 56**	**Day 90**
FSMM1	4	728.3 ± 30.1	887.5 ± 30.5	911.7 ± 24.5	945.7 ± 26.5
	25	728.3 ± 30.1	857.5 ± 23.5	866.7 ± 30.5	878.7 ± 33.5
FSMM2	4	740.2 ± 32.1	857.5 ± 33.5	867.0 ± 34.0	879.0 ± 35.0
	25	740.2 ± 32.1	847.5 ± 33.5	852.0 ± 33.0	861.0 ± 34.0
FSMM3	4	933.3 ± 35.1	952.5 ± 33.5	965.0 ± 32.0	978.0 ± 34.0
	25	933.3 ± 35.1	922.5 ± 30.5	835.0 ± 32.0	943.0 ± 33.0





Therefore, formula FSMM2 which containing inverted sucrose, tween20, glycerol, sorbitol, banana caramel syrup and parabens considered a well-formulated suspension because it was physically and chemically stable due to its fluidity, no sucrose crystallization growth, more taste and chemical stability.

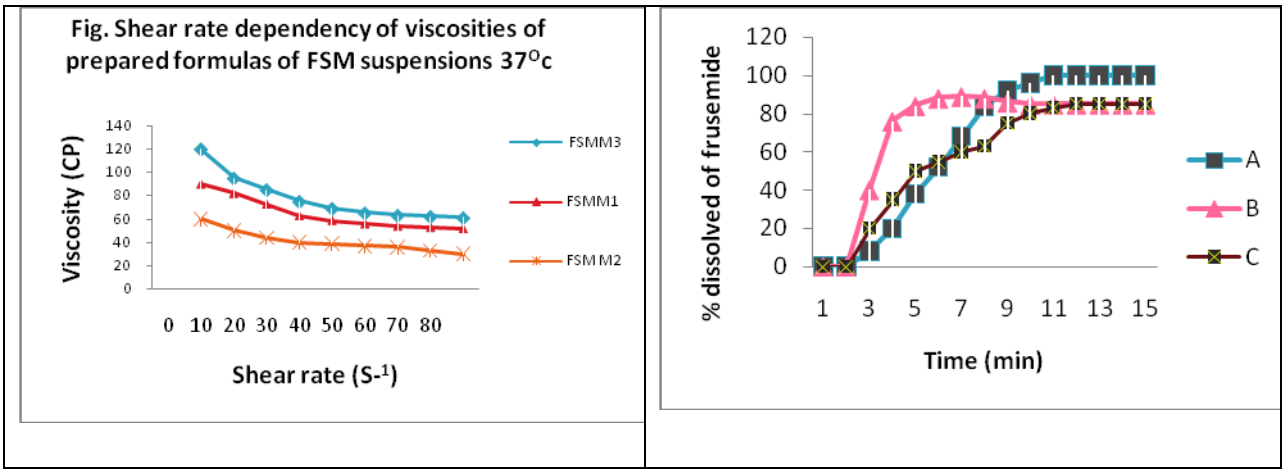


Table 7. Stability of FSM suspensions (in different media vehicles) stored at 4 and 25°C. For two temperatures (n = 3)

Formulation	Temp.(°C)	% Initial concentration remaining*						
		day 0	day 14	Day 28	day 42	day 56	day 72	day 90
FSMM1	4	101.5 ± 1.1	101.1 ± 1.2	100.6 ± 1.8	99.3 ± 2.7	98.8 ± 2.8	97.1± 2.2	97.6± 2.4
	25	101.5± 1.1	101.7± 1.3	100± 1.4	99.6± 2.1	99.2± 2.2	98.4± 2.3	97.6± 2.5
FSMM2	4	103. 4± 1.4	102.1 ± 1.7	101.9 ± 1.5	100.3± 1.6	99.9± 2.8	100.1± 2.1	99.4 ± 2.3
	25	103.4 ± 1.4	102.8 ± 1.5	101.3 ±1.2	100.5 ± 1.3	100.2 ± 1.4	99.8 ± 2.2	98.7± 2.4
FSMM3	4	102.6 ± 1.5	101.8 ± 1.3	100.9 ± 1.5	99.9± 1.8	99.1± 1.9	98.8± 2.5	98.6 ± 2.8
	25	102.6 ± 1.5	101.1± 1.6	100.1 ± 1.7	98..9± 1.9	99..5± 2.2	99..1± 2.3	98.2± 2.5

*The mean values ± S.D.

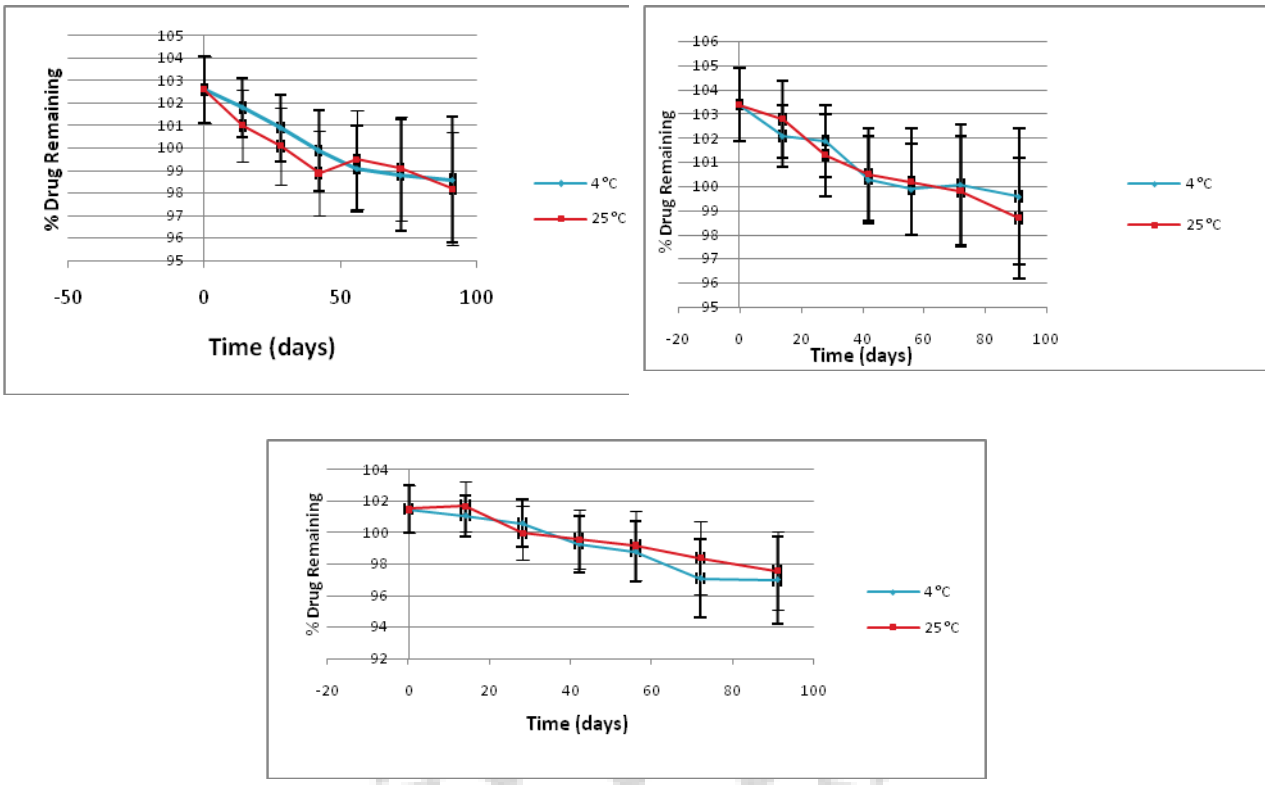


Figure 3 shows the dynamic viscosity as a function of the rotational speed, for samples A and B. Both, the upward and downward curves, presented identical pathways which suggested a pseudoplastic flow without thixotropy. Also, a difference could be observed between formulation A and B viscosity values. Formulation B showed higher viscosity values than formulation A.

Formulation A showed an increase in viscosity values after 28 and 56 days under refrigerated conditions (4^{o}C). However, the samples viscosity was reduced when the storage was performed at 25^{o}C (Table 4) FSMM1 similar trend was followed by formulation FSMM2. Nevertheless, the viscosity variation at 4^{o}C was less marked and values remain almost unchanged. According to the chemical characterization, FSM stability was followed by spectrophotometry. Table 5 shows the initial FSM concentration remaining percentage up to 90 days. Encouraging results were obtained for all formulations as to FSM chemical stability. All Formulations (FSMM1, FSMM2 and FSMM3) presented FSM remaining percentage above 98% for both storage conditions (4 and 25^{o}C).

5.0: CONCLUSION

The aim of the present study was to develop an optimal oral liquid furosemide suspension in order to enhance physical- chemical stability of the drug and paediatric patient acceptance.

As it is well known, suspensions are thermodynamically unstable systems. Therefore, suspended particles would sediment at a certain sedimentation velocity. That is the main reason why they have to be shaken before use, to ensure content uniformity[19].

According to the Stocks Law[23], changes on viscosity values could modify sedimentation velocity of the suspended particles. Generally, suspending vehicles are used to maintain dispersed particles homogeneously distributed in the formulation by keeping an appropriate viscosity. Our formulations presented a pseudoplastic flow because the hydrocolloid used as suspending agent was SMC sodium which imparts non-Newtonian properties of the suspension. This is very important because a pseudoplastic dispersion media could support two processes: (1) Retard the sedimentation of small particles, as their apparent viscosities increase under the small stresses associated with sedimentation, and (2) The medium could undergo structural breakdown under the higher stresses involved in shaking and pouring[24]. Although the suspending vehicle content was reduced from 1 to 0.7% w/v. Formulation FSMM3 showed higher viscosity values than others, probably due to the amount of pharmaceutical excipients presented on commercial tablets.

As to our results, refrigerated storage conditions (4°C) produced a slight increase in formulation viscosity leading to a decrement of sedimentation velocity of the suspended particles. Cancela et al[25] observed that the viscosity of suspensions containing SMC decreases when the temperature increases. This behaviour agrees with our results. Therefore, Vs values at 4^{o}C resulted smaller than those for samples storage at room controlled temperature for a period of 56 days.

One of the main objectives of the present work was to investigate furosemide chemical stability under different storage conditions. Both kinds of formulations (A and B) demonstrated an acceptable drug chemical stability where furosemide content remain above 98% at room controlled temperature and refrigerator temperature for a period of 90 days. In conclusion,

paediatric oral liquid furosemide suspensions presented an adequate physical stability in order to keep the furosemide particles homogeneously distributed in the formulation.

Adequate chemical furosemide stability under refrigerated and room controlled temperature conditions are of real importance since furosemide suspensions could easily be distributed and storage at a paediatric hospital without any special storage conditions.

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