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FORMULATION AND EVALUATION OF AQUEOUS PARENTRAL AND ORAL LIQUID FORMULATION OF FRUSEMIDE

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ABSTRACT

The present investigation constituents the formulation and evaluation of aqueous parenteral and oral liquid formulation a poorly water soluble drug. The aim of the present research study was to explore the possibility of employing mixed hydrotropic solubilization technique in the formulation and evaluation of aqueous parenteral and oral liquid formulation of a poorly water soluble drug. In the present study, practically insoluble model drug, furosemide was tried to solubilize by employing the combination of physiologically compatible hydrotropic agents to attempt its parenteral and oral liquid formulations.

KEYWORD: Analytical method, frusemide, solublization studies, liquid syrup formulation, hydrotropic solubilization, parenteral.

INTRODUCTION

Therapeutic effectiveness of a drug depends upon the bioavailability and ultimately upon the solubility of drug molecules. Solubility is one of the important parameter to achieve desired concentration of drug in systemic circulation for desired pharmacological response. Currently, only 8% of new drug candidates have high solubility and permeability. The aqueous solubility of drugs is often a limiting factor in developing the most desirable dosage forms. Many drugs and drug candidates are poorly water-soluble which limit their clinical applications. Increasing number of newly developed drugs are poorly water-soluble and such poor water solubility causes significant problems in producing formulations of a sufficiently high bioavailability with reproducible effects.[1]

Product development scientists often encounter significant difficulties in solving the problem of poor water solubility of drug candidates in the development of pharmaceutical dosage forms. As a matter of fact, more than one third of the drugs listed in the U.S. Pharmacopoeia fall into the poorly water-soluble or water-insoluble categories. It was reported a couple of decades ago that more than 41% of the failures in new drug development have been attributed to poor biopharmaceutical properties, including water insolubility. Water insolubility can postpone or completely halt new drug development, and can prevent the much needed reformulation of currently marketed products.[2]

MATERIAL AND METHODS

The oral liquid dosage form of poorly water-soluble drugs available in market are mostly in the form of solid dosage form like tablets, capsules etc. Liquid oral solutions (syrups) show better availability and quick onset of action in comparison to the tablet dosage form. In the present investigation, the poorly water-soluble drug furosemide has been selected as model drug for formulating its syrup with the help of sodium benzoate, urea, sodium citrate and sodium acetate as model hydrotropic agents. In the present study, the use of mixed hydrotropy has been explored to develop syrup of poorly water-soluble drug by employing the combination of physiologically compatible hydrotropic agents at reduced concentration to provide quick onset of action and better availability (in comparison to tablet dosage form).

On the basis of the results obtained from the solubilization studies the syrup of poorly water-soluble drug furosemide have been developed.

Selection of solubilizing hydrotropic blend

The same hydrotropic blend $BUCA_3$ mentioned in table 26 (chapter 6) was used for developing the syrup formulation.

Selection of sweetening agents

Sweetening agents can be employed either alone or in combination. The best combination can be selected by employing a taste panel. A number of sweetening agents are used to increase the palatability of an oral liquid preparation.

Sorbitol was selected as a bulk sweetener. Because it has a pleasant cooling, sweet taste (50-60% sweetness of sucrose). It is an excellent humectant and texturizing agent also. It is non

cariogenic, slowly absorbed and may be useful to diabetic patients. It does not take part in Maillard reaction.

Saccharin sodium was selected as intense sweetener. It is 300 times sweeter than sucrose. It may be useful in diabetics as it goes directly through the human digestive system without being digested. It decomposes only at low pH (pH 2).

Selection of flavouring agents

Furosemide is having typical organic acid taste that can be easily masked by fruit flavours. So, orange flavour and pineapple flavours were selected for developing syrup formulations.

Optimization of Formulation Variables For Liquid Syrup Formulation

Syrups with formulation variables like different amount of sweetener, combination of sweeteners were designed as shown in table 1. The formula was obtained and observed for taste by employing taste panel shown in table 2. The palatability of the formulation was further improved by the addition of flavouring agents shown in table 3. Evaluation of syrup containing flavouring agent was also carried out using taste panel shown in table 4.

Required amount of hydrotropic agents were dissolved in warm distilled water (45% of the total volume of syrup). Calculated quantity of furosemide was dissolved in the prepared blend. Required amount of desired additive after dissolving in suitable volume of water was added to the prepared drug solution and properly mixed to get the syrup. The taste of prepared syrups was evaluated by employing the taste panel comprising of seven human volunteers.

The formulation codes of syrup formulations were designed where first letter represent the drug, second letter represent the formulation (syrup), third letter represent the serial number, fourth letter represent the flavour and the subscipts again represent the serial number.

Optimization of sweetening agents

Formulated syrups for selection of amount of sweetening agents are shown in table 41.

Table 1: Syrups for selection of sweeteners.

S. No.	Ingredients	Formulation code					
		FS1	FS2	FS3	FS4	FS5	FS6
1.	Furosemide	1 g	1 g	1 g	1 g	1 g	1 g
2.	SB	10 g	10 g	10 g	10 g	10 g	10 g
3.	U	20 g	20 g	20 g	20 g	20 g	20 g
4.	SC	5 g	5 g	5 g	5 g	5 g	5 g
5.	SA	5 g	5 g	5 g	5 g	5 g	5 g
6.	Sorbitol solution (70%)	21.4 ml (15%)	28.5 ml (20%)	28.5 ml (20%)	28.5 ml (20%)	28.5 ml (20%)	28.5 ml (20%)
7.	Saccharin sodium	-	-	0.04%	0.06%	0.08%	0.08%
8.	Chloroform spirit	-	-	-	-	-	0.6 ml
9.	Distilled water (q.s.)	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml

SB = Sodium benzoate, U = Urea, SC = Sodium citrate, SA = Sodium acetate

Taste evaluation of syrups

The taste of formulated syrups FS1, FS2, FS3, FS4, FS5 and FS6 were evaluated by human volunteers. The taste panel comprising of seven volunteers was formed and each individual was allowed to taste 4 ml of sample. The comments given by them (Bitter, Slight sweet, Sweet, Good) were recorded. The observations are recorded in table 2.

Table 2: Taste evaluation of formulated furosemide syrups.

S. No.	Formulation codes	Taste remark of volunteers						
1.	FS1	-	-	-	-	-	-	-
2.	FS2	-	-	-	-	-	-	-
3.	FS3	+	+	+	+	+	+	+
4.	FS4	++	++	+	++	+	++	++
5.	FS5	+++	+++	+++	+++	+++	+++	+++
6.	FS6	+++(*)	+++(*)	+++(*)	+++(*)	+++(*)	+++(*)	+++(*)

Remarks: - (-) = Bitter (+) = Slightly sweet (++) = Sweet (+++) = Good taste (*) = Absence of unpleasant taste of hydrotropic agents

RESULT AND DISCUSSION

Results of taste evaluation showed that sorbitol alone was unable to mask the bitter, typical organic acid taste of furosemide at the 15 and 20% concentration. Instead of using much higher concentration of bulk sweetener sorbitol, combination with an intense sweetener saccharin sodium was tried. Though, the formulation FS5 provided good taste qualities but unpleasant disaggreable taste of hydrotropic agents due to especially urea was present so chloroform spirit was included in the formulation (FS6) which provided sweetened

acceptable oral liquid formulation. Therefore, sorbitol solution (70%), 28.5 ml (20% of formulation) and saccharin sodium (0.08%) as sweeteners and chloroform spirit as desensitizing agent were selected.

Optimization of flavouring agents

The palatability of the above selected formulation was further improved by the addition of flavouring agents as shown in table 3. Selection of flavouring agents is much more empirical. Taste panels can be useful in selecting one of several candidate formulations.

Table 3: Selection of amount of flavouring agents.

Formulation codes	Orange flavour (%)	Pineapple flavour (%)
$FS6O_1$	0.04	-
$FS6O_2$	0.08	-
$FS6O_3$	0.1	-
$FS6P_1$	-	0.04
$FS6P_2$	-	0.08
$FS6P_3$	-	0.1

Taste evaluation of syrups The taste of formulated syrups was further evaluated by human volunteers. The comments given by taste panel (Good, Better, Best) were recorded. The observations are recorded in table 4.

Table 4: Taste evaluation of syrups formulated with flavouring agents.

S. No.	Formulation codes	Taste remark of volunteers						
1.	$FS6O_1$	+	+	+	+	+	+	+
2.	$FS6O_2$	+	+	++	+	++	+	+
3.	$FS6O_3$	+++	+++	+++	+++	+++	+++	+++
4.	$FS6P_1$	+	+	+	+	+	+	+
5.	$FS6P_2$	+	+	++	++	++	+	+
6.	$FS6P_3$	+++	+++	+++	+++	+++	+++	+++

Remarks:- (+) = Good ++ = Better (+++) = Best

Results and discussion: The above evaluation studies showed that the orange flavour and pineapple flavour provide best results at 0.1% concentration.

Optimized syrup formula

The optimized syrup formula based on above studies is shown in table 45.

Table 5: Optimized formula of furosemide oral liquid formulation (Syrup).

S. No.	Ingredients	Formulation code	Formulation code
		$FS6O_3$	**$FS6P_3$**
1.	Furosemide	10 mg/ml	10 mg/ml
2.	SB	10 g	10 g
3.	U	20 g	20 g
4.	SC	5 g	5 g
5.	SA	5 g	5 g
6.	Sorbitol solution (70%)	28.5 ml (20%)	28.5 ml (20%)
7.	Saccharin sodium	80 mg (0.08%)	80 mg (0.08%)
8.	Chloroform spirit	0.6 ml (0.6%)	0.6 ml (0.6%)
9.	Flavouring agent	Orange (0.1%)	Pineapple (0.1%)
10.	Sodium bisulphite	0.1%	0.1%
11.	Distilled water (q.s.)	100 ml	100 ml

SB = Sodium benzoate, U = Urea, SC = Sodium citrate, SA = Sodium acetate

The syrup $FS6O_3$ was clear with pleasant orange taste. The syrup $FS6P_3$ was clear with pleasant pineapple taste.

Formulation of Liquid Syrup Formulation

The syrup was formulated according to the formulation details given in table 45, following the procedure given below.

Step A

Appropriately weighed quantities of hydrotropic agents were taken in a 100 ml volumetric flask and 45 ml of warm distilled water (to rapidize the dissolution process) was added to it. The flask was shaken to dissolve the hydrotropic agents. Then, the weighed amount of bulk drug, furosemide was added and the flask was shaken to solubilize the drug. Required volume of sorbitol solution was added to the prepared drug solution and the flask containing the resulting solution was shaken to get the homogenous solution. Sodium bisulphite (to preclude the chances of oxidation) was added and flask was shaken to ensure its dissolution.

Step B

Saccharin sodium and flavouring agents (orange flavour in case of FS6O3 and pineapple flavour in case of FS6P3) were dissolved in distilled water and added quantitatively to step A solution. Finally, required volume of chloroform spirit was added. Then, flask was set aside for some time. When the temperature was lowered to room temperature, volume was made upto the mark with distilled water. The prepared syrup was shaken to get the homogenous

solution. The syrup was filtered through the filter paper. First few ml of syrup was discarded. Filtered syrup was filled in air tight glass container.

Determination of Ph of Developed Liquid Syrup

The pH of formulated syrups was determined using digital pH meter (Cyber Scan 510, Eutech Instruments Singapore).

Result: The pH of the formulations $FS6O_3$ and $FS6P_3$ was found to be 7.73 and 7.65 respectively.

Determination of Viscosity of The Developed Liquid Syrup

The viscosities of syrups was determined using Ostwald viscometer.

Result: The viscosity was found to be 16.80 centipoise in both the formulations.

Physical and Chemical Stability Testing of Formulated Syrups

The formulated syrups were subjected to physical and chemical stability testing at three different temperatures (ambient, moderate and relatively higher temperature).

Physical stability testing of formulated syrups

The syrups were filled in 10 ml amber coloured glass vials and vials were plugged and sealed. The vials were kept at room temperature, 40°C/75% RH and 55°C. For physical stability studies, the syrups were observed at definite time intervals for colour change and clarity (to observe any turbidity or precipitation). Observations are recorded in table 46-47.

Table 6: Physical Stability Testing Data For Syrup Formulation $FS6O_3$.

Conditions	Time (days)	Physical parameters		
		Colour	Clarity	Precipitation
RTD	0	Slight yellow	Clear solution	No precipitation
RTD	15	Slight yellow	Clear solution	No precipitation
RTD	30	Slight yellow	Clear solution	No precipitation
40°C/75% RH	0	Slight yellow	Clear solution	No precipitation
40°C/75% RH	15	Slight yellow	Clear solution	No precipitation
40°C/75% RH	30	Slight yellow	Clear solution	No precipitation
55°C	0	Slight yellow	Clear solution	No precipitation
55°C	15	Slight yellow	Clear solution	No precipitation
55°C	30	Slight yellow	Clear solution	No precipitation

Table 7: Physical stability testing data for syrup formulation $FS6P_3$.

Conditions	Time (days)	Physical parameters		
		Colour	Clarity	Precipitation
RTD	0	Slight yellow	Clear solution	No precipitation
RTD	15	Slight yellow	Clear solution	No precipitation
RTD	30	Slight yellow	Clear solution	No precipitation
40°C/75% RH	0	Slight yellow	Clear solution	No precipitation
40°C/75% RH	15	Slight yellow	Clear solution	No precipitation
40°C/75% RH	30	Slight yellow	Clear solution	No precipitation
55°C	0	Slight yellow	Clear solution	No precipitation
55°C	15	Slight yellow	Clear solution	No precipitation
55°C	30	Slight yellow	Clear solution	No precipitation

Result and discussion: Both the syrup formulations were found to be unaffected in respect of colour stability. No visual colour change or precipitate was observed in the developed formulations.

8.1.6.2 Freeze Thaw cycling

In case of products that are susceptible to phase separation, loss of viscosity, precipitation and aggregation an extra investigation involving thermal cycling is carried out to establish the influence of temperature variation during distribution. Under this, the packaged product is cycled through temperature conditions that simulate the changes likely to be encountered once the drug product is in distribution.

Procedure for Freeze Thaw cycling testing of syrups

The vials were kept alternately at 40±1°C and 4±1°C for 24 hour each, and shaken everyday for 5 minutes on a touch type vortex mixer. Two vials of formulation were taken, one of which was kept at 40±1°C and the other at 4±1°C for first day, followed by subsequent temperature cycling and shaking as described. After 7-7 such cycles at 4±1°C and 40±1°C (alternately), the vials were observed to check turbidity and precipitation, if any.

Result and discussion: There was no precipitation and no turbidity in syrup formulations at the end of this testing.

Chemical stability testing of syrups

In order to study the chemical stability of syrups, the samples were collected at fifteen days interval upto one month and analyzed by HPLC method given in sec. 7.1.5.3 (chapter 7) to calculate the residual drug content of the syrups. The initial drug content for each formulation

was taken as 100%. The values of percent residual drug for the formulation at different time intervals as well as at different temperatures are noted in table 48.

Table 8: Chemical stability testing data for syrup formulations $FS6O_3$ and $FS6P_3$.

Conditions	Time (days)	Percent residual drug in formulation	
		$FS6O_3$	$FS6P_3$
Room temperature	0	100	100
Room temperature	15	99.27	99.18
Room temperature	30	98.93	98.87
40±2°C/75% RH	0	100	100
40±2°C/75% RH	15	98.99	98.92
40±2°C/75% RH	30	98.62	98.53
55°C	0	100	100
55°C	15	97.56	97.48
55°C	30	95.82	95.76

Result and discussion: The results of chemical stability studies showed that the residual drug content at the end of one month was more than 98% at 25°C in both the formulations. The residual drug content at 30 day time period in the formulation $FS6O_3$ was 98.62% at 40°C and 95.82% at 55°C whereas in formulation $FS6P_3$, 98.53% at 40°C and 95.76% at 55°C, which indicates that the formulations will have long term stability at room temperature.

SUMMARY AND CONCLUSION

The hydrotropic agents sodium benzoate, urea, sodium citrate and sodium acetate were selected for solubilization studies on the basis of solubility enhancement ratio. The solubility enhancement ratio in sodium gluconate and sodium ascorbate were found to be less than 5. Therefore, they were excluded from the study. The solubility determination of drug in hydrotropic solutions was carried out at room temperature. The solubility was increased upto 200.46 fold in 40% sodium benzoate solution, 14.81 fold in 40% urea solution, 11.85 fold in 40% sodium citrate solution and 9.35 fold in 40% sodium acetate solution. Therefore, the solubilizing power of different hydrotropes could be ranked as:

Sodium benzoate > Urea > Sodium citrate > Sodium acetate

From the equilibrium solubility curves of furosemide in 40% hydrotropic solutions it was concluded, that the increase in solubility was not the linear function of the hydrotrope concentration, but there was slow rise in solubility by increasing the hydrotrope concentration.

From the results of solubility determination studies and considering the exhaustive literature survey hydrotropic blend $BUCA_3$ was employed for developing aqueous injection (10 mg/ml) and syrup (10 mg/ml) of furosemide. In each formulation 0.1% w/v sodium bisulphite was added to preclude any possibility of oxidation since it was found to be most suitable anitioxidant. The parenteral formulation was sterilized by filtration through 0.22 µm membrane filter and filled in amber coloured glass vials with nitrogen gas flushing.

The selection of amount of sweeteners and flavourants for syrup formulation was carried out employing the taste panel. Syrup formulations were developed using combination of sorbitol and sodium sachharin as sweeteners together with orange flavour in formulation $FS6O_3$ and pineapple flavour in formulation $FS6P_3$.

All the prepared formulations were subjected to physical stability testing programme at different temperature conditions for a period of one month. The results showed that the formulations were unaffected in respect of colour stability and precipitation on storage at 25°C, 40°C/75% RH and 55°C. No precipitation was observed at the end of the freeze thaw cycling study. Further, the formulations were subjected to chemical stability studies on storage at 25°C, 40°C/75% RH and 55°C for a period of 30 days. The formulations were analyzed by the HPLC method specified in USP 30 at the interval of 15 days. The results showed that there was no appreciable loss of furosemide after one month at 40°C/75% RH and 55°C which suggest the long term stability of the developed formulations at room temperature.

Parenteral formulation was studied for the effect of dilution with 0.9% NaCl and dextrose solution. No visual precipitation or micro crystals on dilution were observed.

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