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Formulation Development of Aqueous Injection of Poorly Soluble Drug Using Mixed Hydrotropic Solubilization Concept and Its Evaluation

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ABSTRACT

Furosemide is a high ceiling diuretic that exhibits antihypertensive activity and also used in treatment of edema associated with congestive heart failure, liver cirrhosis, renal disease, including nephrotic syndrome. It is practically insoluble in water. The effect of hydrotropes such as urea, sodium citrate, sodium benzoate, sodium acetate and blends on the solubility of furosemide was investigated. The enhancement in the solubility of furosemide was up to 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 compared to its solubility in distilled water. The solubility of drug was raised up to 357.87 fold in blend BUC (containing sodium benzoate, urea and sodium citrate in the ratio of 13.3:13.3:13.3) which was about 1.35 times more than the solubility in the blend BU (containing sodium benzoate and urea in the ratio of 20:20). This proved a synergistic enhancement in solubility of a poorly water soluble drug due to mixed hydrotropy. Combination of hydrotropic agents giving synergistic solvent action can minimize the amount of hydrotropic agents employed, minimizing the chances of their individual toxicities. Aqueous injection of furosemide, using the mixed hydrotropic solubilization technique, was developed. The developed formulation was studied for physical and chemical stability. The prepared formulation was unaffected in respect of color stability. There was no color change or precipitate was found in the developed formulation. In the freeze thaw study there was no precipitation and no turbidity in the developed parenteral formulation at the end of the testing. Chemical stability showed that there was no appreciable loss of furosemide in the formulation stored for 30 days at different temperatures. So it can be assumed that the formulation will have sufficient chemical stability at room temperature.

Keywords: Mixed hydrotropy, solubilization, furosemide, aqueous injection, urea, sodium acetate, sodium benzoate, synergistic enhancement.

INTRODUCTION

Hydrotropy is the term originally put forward by Neuburg [1] to describe the increase in the solubility of a solute by the addition of fairly high concentrations of alkali metal salts of various organic acids. Various hydrotropic agents have been used to enhance the aqueous solubility of a large number of drugs. [2-18] Maheshwari and his associates have analyzed a large number of poorly water-soluble drugs by titrimetric and spectrophotometric analysis. [2-18]

Maheshwari has nicely applied the application of hydrotropy in titrimetric and spectrophotometric estimation of a large number of poorly water soluble

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drugs precluding the use of organic solvents [5-18] for example salicylic acid, ketoprofen, aceclofenac, tinidazole, cefixime and hydrochlorthiazide .

Mixed hydrotropic solubilization technique is the phenomenon to increase the solubility of poorly soluble drugs by the addition of more than one hydrotropic agent. [15] Hydrotropic agents used in combination may enhance the solubility of poorly soluble drugs by miraculous synergistic solvent effect in addition to the additive effect. Utilization of this technique in the formulation made of water insoluble drugs can also reduce the concentrations of the individual hydrotropic agents. [16]

The aim of the present research study was to explore the possibility of employing mixed hydrotropic solubilization technique in the formulation of aqueous parenteral formulation of a poorly water soluble drug and to reduce the concentrations of individual solubilizers to minimize the toxic effects of solubilizers. In present investigation furosemide, an antihypertensive high ceiling diuretic was selected as a model drug and it was tried to formulate its aqueous formulations for parenteral and oral use by employing combination of physiologically compatible hydrotropic agents. The formulation was also studied for chemical and physical stability studies.

MATERIALS AND METHODS

Materials

The gift sample of furosemide was provided by M/s IPCA Laboratories Ltd. Ratlam (M.P.). All other chemicals and solvents used were of analytical grade.

Instrument

A Shimadzu UV/V is recording spectrophotometer (Model-UV1700) with 1 cm matched silica cells was employed for spectrophotometric analysis.

Determination of equilibrium solubility

Aqueous solutions of hydrotropic agents (sodium benzoate, urea, sodium citrate, sodium acetate) of known concentrations (10%, 20%, 30%, and 40%) were prepared in distilled water. Sufficient excess amount of furosemide was added to screw capped amber colored glass vials containing fixed volumes (10 ml) of the hydrotropic solutions separately. The vials were shaken mechanically for 12 hours at room temperature in orbital flask shaker (Khera Instruments Pvt. Ltd., Delhi, India). The solutions were allowed to equilibrate for next 24 hours and then centrifuged for 5 minutes at 2000 rpm using a centrifuge (Remi Instruments Limited, Mumbai, India). The supernatants of each vial were filtered through Whatman filter paper # 41. An aliquot of each filtrate was diluted suitably with distilled water and the resulting solutions were analyzed on UV/Visible spectrophotometer (Shimadzu 1700) at 333 nm against respective reagent blank solutions. The solubilities were determined using the regression equations. The solubility of drug in various hydrotropic solutions and the solubility enhancement ratios are presented in Table 1to Table 4.

Solubility enhancement ratio = Solubility in particular hydrotropic solution/ solubility in distilled water

Table 1: Equilibrium solubility data of furosemide and solubility enhancement ratio in sodium benzoate solutions of varying concentrations

Hydrotropic solution codes	Equilibrium solubility of furosemide (% w/v)	Solubility enhancement ratio
B 10%	0.256	23.70
B20%	0.591	54.70
B 30%	1.138	105.37
B 40%	2.165	200.46

(Where B = sodium benzoate)

Table 2: Equilibrium solubility data of furosemide and solubility enhancement ratio in urea solutions of varying concentrations

Hydrotropic solution codes	Equilibrium solubility of furosemide (% w/v)	Solubility enhancement ratio
U 10%	0.049	4.54
U 20%	0.063	5.83
U 30%	0.100	9.25
U 40%	0.160	14.81

(Where U = Urea)

Table 3: Equilibrium solubility data of furosemide and solubility enhancement ratio in sodium citrate solutions of varying concentrations

Hydrotropic solution codes	Equilibrium solubility of furosemide (% w/v)	Solubility enhancement ratio
C 10%	0.015	1.38
C 20%	0.034	3.14
C 30%	0.060	5.55
C 40%	0.128	11.85

(Where C = sodium citrate)

Table 4: Equilibrium solubility data of furosemide and solubility enhancement ratio in sodium acetate solutions of varying concentrations

Hydrotropic solution codes	Equilibrium solubility of furosemide (%w/v)	Solubility enhancement ratio
A 10%	0.013	1.20
A 20%	0.024	2.22
A 30%	0.053	4.81
A 40%	0.101	9.35

(Where A = sodium acetate)

Determination of equilibrium solubility of furosemide in aqueous blends (solutions) of hydrotropic agents

Different aqueous blends (solutions) of hydrotropic agents were made as per the compositions mentioned in Table 6 and Table 7.

For the preparation of the hydrotropic blends, required amounts of hydrotropes were weighed and transferred to the volumetric flask. Distilled water was added and the flask was shaken vigorously to ensure the complete dissolution of hydrotropic agents. Finally, the volume was made up to the mark with distilled water. Then the solutions were filtered using Whatman filter paper # 41 and used for solubilization studies.

Sufficient excess amount of furosemide was added to screw capped amber colored glass vials containing fixed volumes (10 ml) of the hydrotropic solutions, separately. The vials were shaken for 12 hours at room temperature in orbital flask shaker (Khera Instruments Pvt. Ltd., Delhi, India). The solutions were allowed to

equilibrate for next 24 hours and then centrifuged for 5 minutes using a centrifuge (Remi Instruments Limited, Mumbai, India). The supernatants of each vial were filtered through Whatman filter paper # 41. An aliquot of each filtrate was diluted suitably with distilled water and the resulting solutions were analyzed on UV/ Visible spectrophotometer (Shimadzu 1700) at 333 nm against respective reagent blank solutions. The observations are recorded in Table 5-7.

Table 5: Equilibrium solubility data of furosemide in blends of two hydrotropic agents

Blend codes	Hydrotropes (%w/v)				Equilibrium solubility (% w/v)	Solubility enhancement ratio
	B	U	C	A		
BU	20	20	-	-	2.846	263.50
BC	20	-	20	-	2.730	253.61
BA	20	-	-	20	2.414	223.50
UC	-	20	20	-	0.921	85.27
UA	-	20	-	20	0.669	61.94
CA	-	-	20	20	0.078	7.22

(Where B = sodium benzoate, U = urea, C = sodium citrate, A = sodium acetate)

Table 6: Equilibrium solubility data of furosemide in blends of three hydrotropic agents

Blend codes	Hydrotropes (%w/v)				Equilibrium solubility (%w/v)	Solubility enhancement ratio
	B	U	SC	SA		
BUC	13.3	13.3	13.3	-	3.865	357.87
BUA	13.3	13.3	-	13.3	1.875	173.61
BCA	13.3	-	13.3	13.3	0.412	38.14
UCA	-	13.3	13.3	13.3	1.108	102.59

(Where B = sodium benzoate, U = urea, C = sodium citrate, A = sodium acetate)

Table 7: Equilibrium solubility data of furosemide in blends of four hydrotropic agents

Blend codes	Hydrotropes (%w/v)				Equilibrium solubility (% w/v)	Solubility enhancement ratio
	B	U	C	A		
$BUCA_1$	10	10	10	10	1.847	171.08
$BUCA_2$	20	10	5	5	5.275	488.42
$BUCA_3$	10	20	5	5	3.555	329.16
$BUCA_4$	10	5	20	5	2.047	189.53
$BUCA_5$	10	5	5	20	1.508	139.62

(Where B = sodium benzoate, U = urea, C = sodium citrate, A = sodium acetate)

Table 8: Selection of hydrotropic blend for aqueous injection formulation

Blend codes	Volume of vehicle	Amount of sodium benzoate (mg)	Amount of urea (mg)	Amount of sodium citrate (mg)	Amount of sodium acetate (mg)	Furosemide (mg/ml)
$BUCA_2$	2 ml	400	200	100	100	10
$BUCA_3$	2 ml	200	400	100	100	10

(Where B represents sodium benzoate, U represents urea, C represents sodium citrate and A represents sodium acetate.)

Selection of hydrotropic blend to formulate the injections

On the basis of the results obtained from solubility determination studies, blends BUCA2 and $BUCA_3$ were selected. To develop 2 ml of furosemide injection, the amounts of hydrotropic agents that will be administered through each blend was determined as shown in the Table 8. Though the total concentration of hydrotropic blend was 40% in each case and maximum solubility was obtained in the blend $BUCA_2$, but considering an exhaustive literature survey, it was decided to solubilizer furosemide using the hydrotropic blend $BUCA_3$ for developing its aqueous injection (10 mg/ml).

Optimized formula

Table 9: Composition of aqueous injection formulation of furosemide

S. No.	Product code	Ingredients	Prescribed formula	Working formula
1.	$FBUCA_3$	Furosemide	20 mg	1 g
2.		Sodium benzoate	200 mg	10 g
3.		Urea	400 mg	20 g
4.		Sodium citrate	100 mg	5 g
5.		Sodium acetate	100 mg	5 g
6.		Sodium bisulphite	0.1 %	0.1 %
7.		Water for injection	q.s.	q.s.
		Total volume	2 ml	100 ml

Formulation of injection dosage form

Initially, the appropriately weighed amount of hydrotropic agents (mentioned in Table 9) were transferred to the 100 ml volumetric flask containing 60 ml of the water for injection purged with nitrogen gas. The flask was shaken to dissolve the hydrotropic agents. Finally, the volume was made up to the mark with same water for injection. To prepare the aqueous drug solution the calculated quantity of furosemide was transferred to the 100 ml volumetric flask and 80 ml of the prepared hydrotropic blend was added to it. The flask was sonicated to dissolve the drug. After complete dissolution of the drug, sodium bisulphate 0.1 g was added to preclude the chances of oxidation. The flask was shaken to dissolve the added antioxidant. Other excipients like chelating agents, buffering agents were not included in the formulation since they might upset the basic solubility enhancement ratio. Finally, the volume was made up to the mark with the same hydrotropic blend. Flask was shaken to get the homogenous solution. After the preparation of the solution, it was filtered through membrane filter 0.22µm (Millipore, Sartorius Germany).

Treatment of packaging material

Amber colored glass vials were first washed three times with distilled water. Finally, these were washed with distilled water, already passed through 0.45µm membrane filter. All these vials were dried in an oven and sterilized by dry heating in an oven at 160°C for 2 hours in inverted position. Rubber closures and aluminum seals used for plugging the vials were first washed several times with distilled water and then autoclaved (Khera Instruments Pvt. Ltd., Delhi, India) at 15 lbs pressure (121°C) for 20 minutes and finally dried in oven.

Preparation of aseptic area

The walls and floor of aseptic room were thoroughly washed with filtered tap water followed by 5% phenol

solution. The laminar airflow bench (Khera Instruments Pvt. Ltd., Delhi, India) was scrubbed with 70% isopropyl alcohol and the HEPA filter and UV light were switched on an hour before the filling of injection into the vials.

Aseptic Filtration

Membrane filter 0.22µm (Millipore, Sartorius Germany) was used for the filtration. The membrane filtration assembly fitted with the membrane filter was sterilized previously in the autoclave (Khera Instruments Pvt. Ltd., Delhi, India) at 121°C and 15 lbs pressure for 20 minutes.

Final flushing with nitrogen gas

The sterile vials were pre- and post- flushed with sterile nitrogen gas, filled with 2 ml volumes of sterile aqueous solution of furosemide, stoppered immediately and sealed with sterile aluminum caps.

Stability Studies

Physical stability testing

The sealed vials of the prepared formulation were visually inspected every 15 days for 45 days against black and white backgrounds to see the changes occurring, if any, in physical appearance of aqueous injection like color, clarity, precipitation, etc. These studies were carried out under room temperature in dark (R.T.D.) and other temperatures (at 40°C/75% RH and 55°C).

Freeze Thaw Cycling (FTC)

This method was designed to simulate storage and temperature conditions and to induce any anticipated precipitation and check it in a much shorter time. The vials were kept alternately at 40± 1°C and 4±1°C for 24 hour each, and shaken every day 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.

Chemical stability testing

Furosemide formulation was further subjected to chemical stability testing at room temperature, 40°C/75% RH (as per ICH guideline), and at 55°C for a period of one month. The samples were analyzed by the HPLC method initially and at fifteen days intervals up to one month to calculate the residual drug content. The initial drug content for the formulation was taken as 100%.

Dilution profiles of aqueous injection formulation

The effect of dilution with intravenous fluids (normal saline solution and 5% dextrose solution) was studied on the prepared formulation shown in Table 10.

Test dilutions of furosemide aqueous formulation in different vehicles of different concentrations, were prepared in thoroughly cleaned and dried volumetric flasks with normal saline and 5% dextrose solution. Dilutions were made in duplicate at room temperature. The prepared dilutions (1:1 to 1:100) were examined visually for the presence of visible precipitate or microcrystals using a sample of intravenous fluid for comparison.

Table 10: Precipitation of furosemide in developed formulation after dilution with normal saline and 5% dextrose solution

Dilution	Time (hour)													
	Normal saline							5% Dextrose solution						
	0.5	1	2	3	6	8	24	0.5	1	2	3	6	8	24
1:1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:20	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:30	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:40	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:50	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:100	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 11: Physical stability data for furosemide injection $FBUCA_3$

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

Table 12: Chemical stability data for furosemide injection $FBUCA_3$

Conditions	Time (days)	Percent residual drug in formulation
		$FBUCA_3$
Room temperature	0	100.00
Room temperature	15	99.47
Room temperature	30	98.87
40°C/75% RH	0	100.00
40°C/75% RH	15	98.86
40°C/75% RH	30	96.25
55°C	0	100.00
55°C	15	97.12
55°C	30	94.85

RESULTS AND DISCUSSION

The solubility determination of furosemide was carried out in distilled water, hydrotropic solutions (40% urea, 40% sodium citrate, 40% sodium acetate, 40% sodium benzoate) and solutions containing different concentrations of hydrotropic agents (urea, sodium benzoate, sodium acetate and sodium citrate). The results of solubility studies are presented in Table 1-7.

It seems from the results that the aqueous solubility of furosemide was increased more than 488.42 times in hydrotropic blend BUCA2, 329. 16 times in hydrotropic blend $BUCA_3$ and up to 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. It is concluded that the solubility of furosemide increases synergistically by mixed hydrotropy. In order to reduce the concentration of sodium benzoate we had used the formulation $BUCA_{3.}$ To find out the influence of pH on solubility of furosemide, the solubility of furosemide was determined in the buffer solution of pH 8.0, 8.4 and 9.0. The results indicated that the enhancement in solubility of the drug was not entirely due to pH effect but mostly due to hydrotropic solubilization phenomenon.

The results of physical stability study (Table 11) showed that the prepared formulation was unaffected in respect of color stability. No visual color change or precipitate was revealed in the developed formulation.

The results of freeze thaw study showed that there was no precipitation and no turbidity in the developed parenteral formulation at the end of the testing.

The results of chemical stability study (Table 12) showed that there was no appreciable loss of furosemide in the formulation stored for 30 days at different temperatures. So it can be assumed that the formulation will have sufficient chemical stability at room temperature.

In conclusion, the findings of this study suggest that a stable aqueous injection of furosemide has been developed. This study further opens the chance of preparing such injection for many other poorly water soluble drugs, using the concept of mixed-hydrotropy. In this way, the toxicity issues related to the hydrotropic solubilizers used were minimized as the individual concentration of solubilizers in blend was reduced. The proposed techniques would be economic, convenient and safe. Thus, this study opens the chance of preparing aqueous formulations of poorly–water soluble drugs, employing the mixed- hydrotropy technique.

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