WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 13, 1391-1411. Research Article ISSN 2277– 7105

SOLUBILITY ENHANCEMENT OF POORLY WATER-SOLUBLE DRUG NIFEDIPINE FOR PARENTERAL ROUTE OF ADMINISTRATION

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Article Received on
01 Sept. 2021,

Revised on 21 Sept. 2021,
Accepted on 11 October 2021

DOI: 10.20959/wjpr202113-22011

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ABSTRACT

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 of a poorly water-soluble drug. In the present study, slightly water-soluble drug, nifedipine was tried to solubilize by employing the combination of physiologically compatible hydrotropic agents to attempt its parenteral and oral liquid formulations. The aqueous solubility of a non-electrolyte can be increased by a variety of techniques. The first step in selecting a method of solubilization is to determine why the drug is insoluble. The easiest way to determine whether DD is a determinant of the drug's insolubility is to look at its melting point. If MP > 200°C, DD is probably a factor significant in reducing the solubility whereas if MP > 300°C, DD is a major factor. As stated above, each 100°C increase in melting point above 25°C corresponds to at least a 10-fold decrease in solubility. If the drug is a liquid or if it melts below 100°C, it is not likely that crystal interactions have any significant effect on solubility. Nifedipine, the prototype of the dihydropyridine class of calcium channel blockers (CCBs), is similar to other dihydropyridines including amlodipine, felodipine, isradipine, and nicardipine. There are at least five different types of calcium channels in Homo sapiens: L-, N-, P/Q-, R- and T-type. CCBs target L-type calcium channels, the major channel in muscle cells that mediates contraction. Similar to other DHP CCBs, nifedipine binds directly to inactive calcium channels stabilizing their inactive conformation. Since arterial smooth muscle depolarizations are longer in duration than cardiac muscle depolarizations, inactive channels are more prevalent in smooth muscle cells.

KEYWORDS: Solubilization, Nifedipine, Calcium Channel Blockers, Solubility enhancers, Phase solubility analysis, Excess solute method.

INTRODUCTION

Therapeutic effectiveness of a drug depends upon the bioavailability and ultimately upon the solubility of drug molecules. Solubility is one of the important **parameters** 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]

In pharmaceutical science, solubility is commonly related to the bioavailability of the compound of interest, especially for poorly soluble compounds. Slow absorption rate result in an erratic and variable profile of drug level. Administration of a drug in any dosage form, except solution involves a dissolution step. Thus, it is required that the drug has to be present in the dissolved state at the site of absorption and then only it can be absorbed. In general, in order for a drug to exert its biological effect, it must be soluble in and transported by the body fluids, transverse the required biologic membrane barriers, escape widespread distribution to unwanted areas, endure metabolic attack, penetrate inadequate concentration to the sites of action and interact in a specific fashion, causing an alteration of cellular function.[3] Solubility is defined in quantitative terms as the concentration of the solute in a saturated solution at a certain temperature and in the qualitative terms it may defined as the spontaneous interaction

of two or more substances to form a homogenous molecular dispersion. A saturated solution is one in which the solute is in equilibrium with the solvent.[4]

Table 1: Solubility concentration expressions.[5]

Expressions	Symbol	Definition
Molarity	M	Moles of solute in 1 litre of solution
Normality	N	Gram equivalent weights of solute in 1 litre of solution
Molality	m	Moles of solute in 1000 g of solvent
Mole fraction	X	Ratio of the moles of one constituent of a solution to the total moles of all constituents
Mole's percent	-	Moles of one constituent in 100 moles of the solution
Percent by weight	% w/w	Grams of solute in 100 grams of solution
Percent volume-in-volume	% v/v	Milliliters of solute in 100 ml of solution
Percent weight-in-volume	% w/v	Grams of solute in 100 ml of solution
Milligram percent	-	Milligrams of solute in 100 ml of solution

For substances whose solubilities are not definitely known, the values are described in pharmaceutical compendia by the use of certain general terms, as given in table 2. The U.S. Pharmacopoeia and National Formulary list the solubility of drugs as the number of milliliters of solvent in which 1 gram of solute will dissolve. For example, the solubility of boric acid in 18 ml of water is given in the U.S. Pharmacopoeia as follows: 1 g of boric acid dissolves in 18 ml of water, in 18 ml of alcohol, and in 4 ml of glycerin.

Table 2: Terms of approximate solubility.[6]

Term	Parts of solvent required for one part of solute
Very soluble	Less than one
Freely soluble	1 to 10
Soluble	10 to 30
Sparingly soluble	30 to 100
Slightly soluble	100 to 1000
Very slightly soluble	1000 to 10,000
Practically insoluble or insoluble	More than 10,000

Methods of solubility determination[7]

No universally acceptable method for solubility determination is known. Solubility of solids in liquids may be determined by:

1. **Phase solubility analysis** - This technique is especially applicable to determining the solubility of substances and also detecting the presence of impurities. In this method, successively larger portions of the substance are added to the same volume of solvent in

suitable containers which are agitated at constant temperatures, generally 30±0.1°C. In those containers in which excess drug is present (undissolved), samples of the supernatant are withdrawn and assayed until the concentration is constant (i.e., the system has reached equilibrium). The solubility is readily determined by extrapolating the line with a slope of zero to the Y axis.

2. **Excess solute method -** The equilibrium solubility of the drug candidate is obtained by equilibrating an excess of material in a vial with the solvent. The vial is shaken at constant temperature and the amount of drug is determined periodically by analysis of the supernatant until the equilibrium has been achieved.

Solubility determination of organic molecules having very low solubilities is hampered by such problems as slow equilibration during measurement, influence of impurities, and inherent heterogeneity in the energetic content of the crystalline solid. Higuchi et al. have described methods for determining solubility of barely aqueous soluble organic solids (solubility less than 0.2%). The three methods mentioned are:

1. Enhancing the dissolution rate of the substance by addition of a water immiscible solvent in which the substance is more soluble, thereby increasing the surface area available for dissolution.
2. Measurement of solubility in an organic solvent and calculation of the aqueous solubility from the estimated partition coefficient and the organic solvent data.
3. Using an excess of the solid and a highly specific analytical technique.

Solubilization[8]

The aqueous solubility of a non-electrolyte can be increased by a variety of techniques. The first step in selecting a method of solubilization is to determine why the drug is insoluble. The easiest way to determine whether DD is a determinant of the drug's insolubility is to look at its melting point. If MP > 200°C, DD is probably a factor significant in reducing the solubility whereas if MP > 300°C, DD is a major factor. As stated above, each 100°C increase in melting point above 25°C corresponds to at least a 10-fold decrease in solubility. If the drug is a liquid or if it melts below 100°C, it is not likely that crystal interactions have any significant effect on solubility. For liquids and low melting drugs the most fruitful techniques of solubilization are those that alter the solvent in such a way as to decrease DD + W′W′ - 2DW′, where W′ represents the aqueous phase. Surfactants, cosolvents and soluble

complexing agents can be used to decrease W′W′ or to increase DW′. In other words, they make the aqueous phase a more favorable environment for the drug.

Methods to enhance the solubility of a drug[9]

In pharmaceutical field, it is often required to prepare aqueous solutions of a variety of insoluble drugs. The ability to increase the aqueous solubility can be a valuable aid for increasing the efficacy and/or reducing adverse effects for certain drugs. Following approaches can be employed to enhance the aqueous solubility of poorly soluble drugs.

- Alteration of pH
- Use of cosolvents
- Effect of dielectric solvent
- Use of surface-active agents
- Complexation
- Hydrotropic solubilization
- Chemical modification of the drug

The above-mentioned approaches have been used widely in various fields of pharmacy. However, applications of 'Mixed Hydrotropic Solubilization' have not been explored to appreciable extent in various fields of pharmacy.

Hydrotropic solubilization

Additives may either increase or decrease the solubility of a solute in a given solvent. These salts that increase solubility are said to '**salt in'** the solute and those salts that decrease the solubility '**salt out'** the solute. The effect of an additive depends very much on the influence, it has on the structure of water or its ability to compete with the solvent water molecules.[10]

Several salts with large anions or cations which are themselves very soluble in water result in a salting in of non-electrolytes called 'Hydrotropic Salts' a phenomenon known as 'HYDROTROPISM'.[11]

These compounds may be anionic, cationic or neutral molecules. However, the term has been used in the literature to designate non-micelle forming substances either liquids or solids, organic or inorganic capable of solubilizing insoluble compounds.[12]

At concentrations higher than a minimal hydrotrope concentration, hydrotropic agents self-associate and form noncovalent assemblies of lowered polarity, i.e., nonpolar microdomains, which solubilize hydrophobic solutes. The self-aggregation of hydrotropic agents is different from surfactant self-assemblies (i.e., micelles) in that hydrotropes form planar or open-layer structures instead of compact spheroid assemblies. Hydrotropic agents are structurally characterized by having a short, bulky, compact moiety such as an aromatic ring, while surfactants are characterized by long hydrocarbon chains. In general, hydrotropic agents have a shorter hydrophobic segment, leading to higher water solubility, than do surfactants.[13]

Advantages of hydrotropic solubilization[14]

1. Hydrotropy is suggested to be superior to another solubilization methods, such as micellar solubilization, miscibility, solvency and salting in, because the solvent character is independent of pH, has high selectivity and does not require emulsification. It only requires mixing the drug with the hydrotrope in water.
2. It does not require chemical modification of hydrophobic drugs, use of organic solvents, or preparation of emulsion system.
3. Economic, safe and user-friendly method.

MATERIALS AND METHOD

Chemicals: Nifedipine was obtained as a generous gift sample from Ranbaxy, Mumbai, sodium benzoate, urea, sodium citrate, sodium acetate were purchased from Central drug house Pvt. Ltd., New Delhi. Polyethylene Glycol (PEG 400) was purchased from Merck India Ltd. Distilled water prepared using glass distillation unit was used throughout the study.

Identification and Characterization of drug

Determination of melting point

The melting point of nifedipine was determined using open capillary method. The capillary filled with drug powder was placed in Thiele's tube containing liquid paraffin. The tube was heated and the melting point of drug powder was noted.

Infrared spectroscopy of nifedipine

The infrared spectroscopy of nifedipine was performed for identification of drug. About 1 mg of the sample was placed over the probe of the FTIR spectrophotometer. The IR spectra is presented in fig.1.

UV Spectroscopic analysis of nifedipine

Drug solution of concentration 0.0005% w/v in 0.1 M HCl was prepared and scanned in the range of 200-400 nm on double beam UV/Visible spectrophotometer. Spectrum of nifedipine is shown in the fig. 2.

Determination of drug content

Weigh accurately about 0.13 g, dissolve in a mixture of 25 ml of *2-methyl-2-propanol* and 25 ml of 1M perchloric acid and titrate with 0.1M ceric ammonium sulphate using 0.1 ml of ferroin solution as indicator until the pink colour is discharged, titrating slowly towards the end-point. Perform a blank determination and make any necessary correction.

Solubilization studies

Determination of solubility comprises of preparing a saturated solution of the given substance and finding out the amount present in a definite quantity of the solution. Rapid solution can be obtained by constant agitation of the solvent and an excess amount of the drug substance. After a given period of stirring, the clear solution is analyzed. The result is taken as the solubility at that particular temperature.

Preparation of calibration curve of nifedipine in distilled water

50 mg of nifedipine bulk drug was accurately weighed and transferred to a 500 ml volumetric flask. About 450 ml of distilled water was added and flask was shaken vigorously to dissolve the drug. After complete dissolution of drug, the volume was made up to the mark with distilled water. Flask was shaken to produce a homogenous solution (stock solution). This stock solution was suitably diluted with distilled water to give various standard solutions containing 5, 10, 15, 20 and 25 µg/ml of drug. The absorbances of these solutions were measured on double beam UV/Visible spectrophotometer at 238 nm. The absorbances data obtained from various concentrations were subjected to linear regression analysis. The observations are recorded in the table 5 and graphically represented in fig. 3.

Determination of equilibrium solubility of nifedipine in distilled water

Sufficient excess amount of nifedipine was added to screw capped amber coloured glass vials containing 10 ml of distilled water. The vials were shaken mechanically for 12 hours at room temperature in orbital flask shaker. The solutions were allowed to equilibrate for next 24 hours and then centrifuged for 5 minutes at 2000 rpm using a centrifuge. The supernatants of

each vial were filtered through Whatman filter paper # 41. An aliquot of each filtrate was diluted suitably with distilled water and analyzed spectrophotometrically at 328 nm.

Solubilization study of nifedipine in hydrotropic solutions

Selection of hydrotropic agents for nifedipine

25 ml of hydrotropic solution was taken in a 50 ml glass bottle and gross weight (including the cap) was noted. Then, few mg (by visual observation) of fine powder of drug was transferred to the bottle. This bottle was shaken vigorously (by hand). When drug got dissolved more drug (few mg by visual observation) was transferred to the bottle and again the bottle was shaken vigorously. Same operation was repeated till some excess drug remained undissolved (after constant vigorous shaking for 10 minutes). Then again gross weight was noted. From the difference in two readings (of weight), an approximate solubility was determined and solubility enhancement ratios (solubility in hydrotropic solution/solubility in distilled water) were calculated. When the solubility enhancement ratio determined was at least 5, such hydrotropic solution was selected for the drug for further studies.

Determination of interference of hydrotropic agents in spectrophotometric estimation of nifedipine

The powders of four different hydrotropic agents sodium benzoate, urea, sodium citrate and sodium acetate were dried in vacuum for 24 hours prior to use and stored in well closed containers. The solutions of each hydrotropic agents of known concentration 1000 µg/ml in distilled water were prepared and scanned on UV/Visible spectrophotometer against same reagent solution in the region from 200-400 nm. The cut off wavelength (nm) and corresponding absorbances are given in the table 6.

Preparation of calibration curve of nifedipine in presence of hydrotropic agents/hydrotropic blends

50 mg of nifedipine bulk drug was accurately weighed and transferred to a 100 ml volumetric flask. Sufficient volume of 40% sodium benzoate solution was added and drug was dissolved in this solution. After complete dissolution of drug, sufficient distilled water was added to make up the volume. The flask was shaken to produce a homogenous stock solution. This stock solution was further diluted with distilled water to get various standard solutions containing 20, 40, 60, 80 and 100 µg/ml of drug. The absorbances of these solutions were measured on UV/Visible spectrophotometer at 328 nm against respective reagent blanks. The

values of absorbances of standard solutions were used to obtain regression equation for the estimation of nifedipine in presence of sodium benzoate.

Similar procedure was repeated using 40% urea solution, 40% sodium citrate solution, 40% sodium acetate solution and hydrotropic blends to obtain the regression equation for the estimation of drug in their presence. Regression equations are shown in table 7.

Determination of equilibrium solubility of nifedipine in different hydrotropic agent solutions

Aqueous solutions of hydrotropic agents (sodium benzoate, urea, sodium citrate, sodium acetate) of known concentrations (10%, 20%, 30%, 40%) were prepared in distilled water.

Sufficient excess amount of nifedipine was added to screw capped amber coloured 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. The solutions were allowed to equilibrate for next 24 hours and then centrifuged for 5 minutes at 2000 rpm using a centrifuge. 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 at 328 nm against respective reagent blank solutions. The solubilities were determined using the corresponding regression equations given in table 7. The solubility of drug in 10%, 20%, 30% sodium benzoate solution, urea solution, sodium citrate solution and sodium acetate solution were determined using regression equation of corresponding 40% solution. The solubility enhancement ratios were also calculated.

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

The observations are recorded in table 8 are shown graphically in fig. 4.

Determination of equilibrium solubility of nifedipine in blends of hydrotropic agents:

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 upto the mark with distilled water. Then the solutions were filtered using Whatman filter paper # 41 and used for solubilization studies.

The method to determine solubility enhancement reported in previous section was used. The solubility enhancement ratios were also calculated. The observations are recorded in table 9-10.

Determination of pH of blends of hydrotropic agents

The pH of the selected hydrotropic blends was determined using digital pH meter. The results are shown in table 11.

Determination of equilibrium solubility of nifedipine at pH corresponding to pH of blends of hydrotropic agents

The selected blends of hydrotropic agent have the pH in the range of 8.0-9.0. So solubility of drug was determined in this pH range and also at physiological pH 7.4. The buffer solutions of pH 7.4, 8.0, 8.4 and 9.0 were prepared and pH of these solutions was measured using digital pH meter. The method to determine solubility enhancement reported in previous section was used. The calculated solubility values and solubility enhancement ratios are recorded in table 12.

Development of aqueous parenteral formulation

The present investigation was proposed to solubilize nifedipine using combination of various physiologically compatible hydrotropic agents. By increasing the solubility of the drug, it might be possible to formulate the small volume parenteral, which will be useful in patients with acute pulmonary oedema and cerebral oedema in which the intravenous administration of nifedipine is preferred.

Selection of hydrotropic blend

On the basis of the results obtained from solubility determination studies, blends $BUCA_2$ and $BUCA_3$ were selected. To develop 2 ml of nifedipine injection, the amount of hydrotropic agents that will be administered through each blend was determined as shown in the table 13. 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 solubilize nifedipine using the hydrotropic blend $BUCA_3$ for developing its aqueous injection (10 mg/ml).

Selection of antioxidant

In order to select proper antioxidant, the calculated quantity of nifedipine and the desired antioxidant in its specified concentration were dissolved in 80 ml of the selected blend $BUCA_3$ in 100 ml volumetric flask. Finally, the volume was made upto the mark using the same blend. The prepared solutions were transferred to the amber-coloured glass vials and subjected to the stability studies at 40°C after the vials being capped and sealed. The colour and clarity of the prepared solutions were examined and the percent residual drug content was estimated after 2 days, 3 days and 5 days using the regression equation Y = 0.0147x + 0.0113 as given in table 7. The results are shown in the table 14.

Formulation of optimized aqueous injection

Initially, the appropriately weighed amount of hydrotropic agents (table 15) was 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 upto the mark with same water for injection. To prepare the aqueous drug solution the calculated quantity of nifedipine 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 bisulphite 0.1% 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 upto 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 0.22 µm.

Determination of pH optimized aqueous injection

The pH of prepared formulation was determined using digital pH meter.

Stability monitoring

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 colour, clarity, precipitation, etc. These studies were carried out under following storage conditions:-

Room temperature in dark (R.T.D.) - This involved keeping of vials at room temperature in dark place.

Other temperatures - The vials were also kept at 40°C/75% RH and 55°C.

The observations are recorded in table 16.

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.

IN- VITRO Evaluation

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 17.

Test dilutions of nifedipine 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.

RESULTS

Melting point: The melting point of nifedipine was found to be **173-175°C**.

IR- Spectra

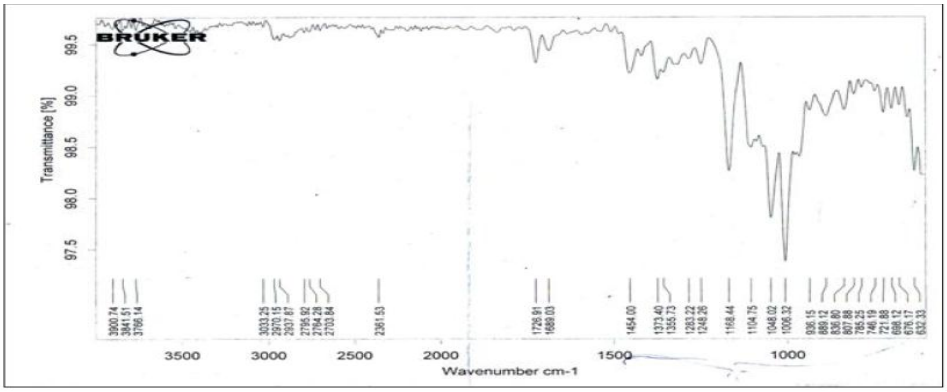

Fig. 1: IR spectra of nifedipine.

UV Spectra: The prepared nifedipine drug solution exhibited maxima at about **238 nm.**

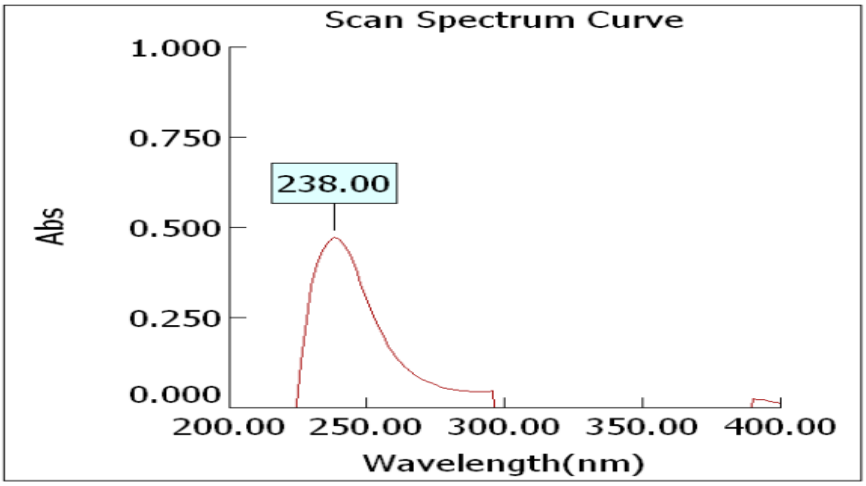

Fig. 2: UV spectra of nifedipine.

Drug content: The drug content in nifedipine bulk drug sample was found to be **99.77%.**

Solubilization studies

Table 5: Absorbance data for calibration curve of nifedipine in distilled water at 238 nm.

S. No.	Concentration (µg/ml)	Absorbance (mean±S.D.) (N=3)
1.	5	0.155 ± 0.0018
2.	10	0.325 ± 0.0014
3.	15	0.485 ± 0.0021
4.	20	0.659 ± 0.0021
5.	25	0.826 ± 0.0025

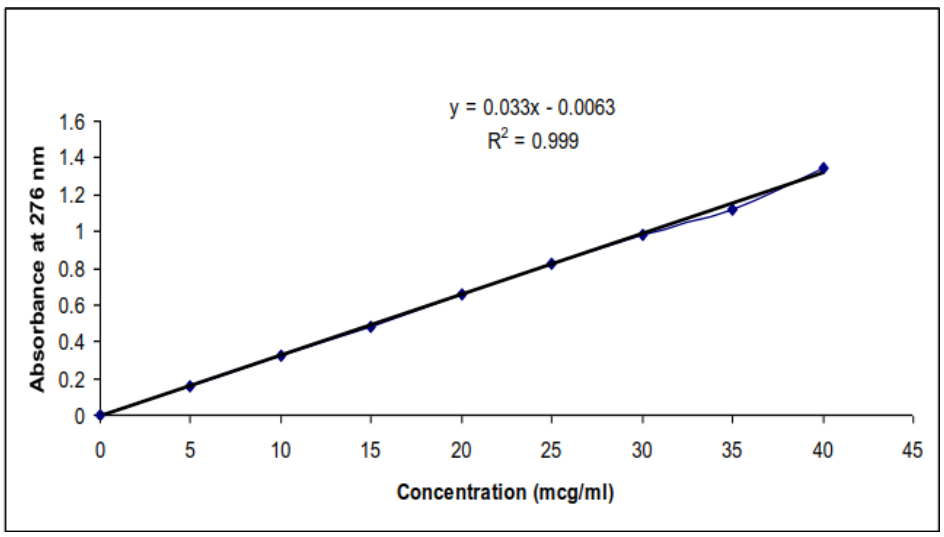

Fig. 3: Calibration curve of nifedipine in distilled water at 238 nm.

The observed solubility of nifedipine was **0.0108% w/v**.

Solubilization study in Hydrotropic Solution

Table 6: UV cut-off wavelength of hydrotropic agents.

S. No.	Hydrotropic agents	Cut-off wavelength (nm)	Absorbance
1.	Sodium benzoate	278.6	0.008
2.	Urea	225.2	0.005
3.	Sodium citrate	245.0	0.020
4.	Sodium acetate	279.0	0.005

It is evident from table 6 that the cut-off wavelength for each of the selected hydrotropes is less than 300 nm which indicates that they do not interfere in the spectrophotometric estimation of nifedipine at 328 nm.

Calibration curve of nifedipine in the presence of Hydrotropic agents/ Hydrotropic Blends

Table 7: Regression equations used for the determination of solubilities in hydrotropic solutions and hydrotropic blends.

S. no.	Hydrotropic solution/blend codes	Regression equations	Regression coefficients (R^2)
1.	B 40%	0.0121x + 0.0052	0.9999
2.	U 40%	0.0120x + 0.0050	0.9994
3.	C 40%	0.0127x – 0.0021	0.9999
4.	A 40%	0.0149x – 0.0168	0.9997
5.	$BUCA_1$	0.014x – 0.0213	0.9989
6.	$BUCA_2$	0.0153x + 0.0101	0.9998
7.	$BUCA_3$	0.0128x + 0.0106	0.9990
8.	$BUCA_4$	0.014x + 0.0148	0.9990

B-Sodium benzoate, U-Urea, C-Sodium citrate, A-Sodium acetate.

Determination of equilibrium solubility of nifedipine in different hydrotropic agent solutions

It is evident from the results that solubility of nifedipine increased with increasing concentration of hydrotropic agents in a nonlinear fashion. The solubilizing power of different hydrotropic agents could be ranked as:

Sodium benzoate > Urea > Sodium citrate > Sodium acetate

The solubility enhancement ratio was found to be highest in hydrotropic solutions of 40% concentration but such a high concentration of individual hydrotropic agents is not preferable, so the combinations of these agents were tried systematically taking overall strength of hydrotropic solution to be 40% to enhance the solubility of nifedipine.

Table 8: Equilibrium solubility of Nifedipine and Solubility enhancement ratio in hydrotope solutions of varying concentrations.

Hydrotropic solution	Equilibrium solubility of nifedipine (% w/v)	Solubility enhancement ratio
B 10%	0.256	21.70
B 20%	0.591	51.70
B 30%	1.138	101.37
B 40%	2.165	197.46
U 10%	0.049	4.04
U 20%	0.063	5.23
U 30%	0.100	8.95
U 40%	0.160	14.44
C 10%	0.015	1.01
C 20%	0.034	3.04
C 30%	0.060	5.25
C 40%	0.128	10.85
A 10%	0.013	0.80
A 20%	0.024	1.67
A 30%	0.053	4.43
A 40%	0.101	9.06

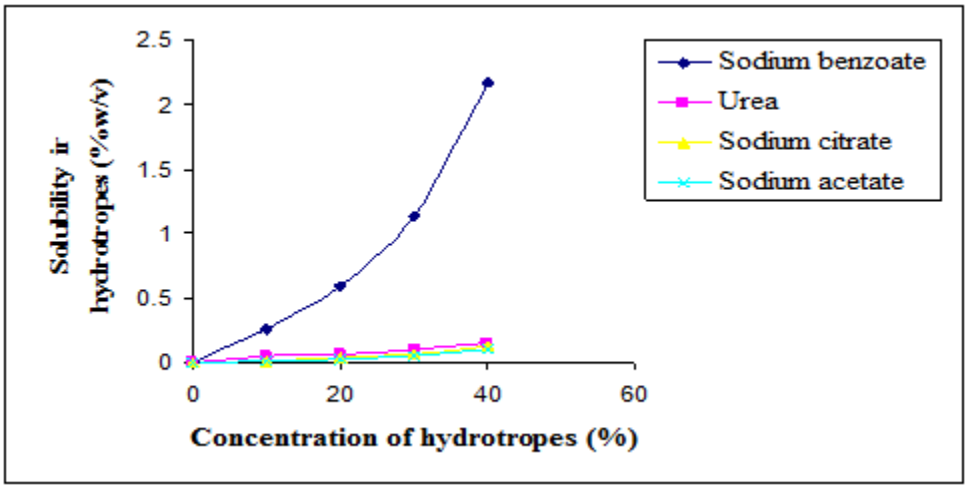

Fig. 4: Comparative equilibrium solubility curves of nifedipine in various hydrotropic solutions.

Determination of equilibrium solubility of nifedipine in blends of hydrotropic agents

Results showed that the solubility of nifedipine in blend containing four agents in equal concentration was increased upto 141.08 fold. The highest enhancement in solubility of nifedipine was obtained in blend $BUCA_2$ (468.12 fold). Synergistic effect of hydrotropic agents has been observed when used in combinations to solubilize the nifedipine. Since the

hydrotropic blends $BUCA_2$ and $BUCA_3$ provided maximum enhancement in solubility at reduced individual agent concentration so these two blends were selected for further studies.

Table 9: Equilibrium solubility data of nifedipine in blends of four hydrotropic agents.

Blend codes	Hydrotropes (%)				Equilibrium solubility (% w/v)
	B	U	C	A	
$BUCA_1$	10	10	10	10	1.487
$BUCA_2$	20	10	5	5	4.985
$BUCA_3$	10	20	5	5	3.275
$BUCA_4$	10	5	20	5	2.011

Table 10: Solubility enhancement ratio in blends of four hydrotropic agents.

Blend codes	Solubility enhancement ratio
$BUCA_1$	141.08
$BUCA_2$	468.12
$BUCA_3$	301.03
$BUCA_4$	179.35

Determination of pH of blends of hydrotropic agents

Table 11: pH of selected blends of hydrotropic agents.

S. No.	Blend codes	pH
1.	$BUCA_2$	8.27
2.	$BUCA_3$	8.11

Determination of equilibrium solubility of nifedipine at pH corresponding to pH of blends of hydrotropic agents

The solubility was increased upto 0.549% w/v (53.26 fold) at pH 9.0 as compared to 4.985% w/v (468.12 fold) and 3.275% w/v (301.03 fold) in hydrotropic blends $BUCA_2$ and $BUCA_3$ respectively. Thus, it can be said that, the enhancement in solubility of nifedipine with hydrotropic blend was not entirely due to pH effect but mostly due to hydrotropic solubilization phenomenon.

Table 12: Equilibrium solubility data of Nifedipine and Solubility enhancement ratio in buffers.

S. no.	Buffer (pH)	Solubility (% w/v)	Solubility enhancement ratio
1.	7.4	0.136	11.28
2.	8.0	0.151	13.91
3.	8.4	0.227	20.07
4.	9.0	0.549	53.26

Selection of Hydrotropic Blend and Antioxidant for aqueous injection

Table 13: Selection of hydrotropic blend for aqueous injection formulation.

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

Table 14: Effect of antioxidant on the stability of nifedipine solubilized product.

Antioxidant	Colour observation			Clarity observation			Percent residual drug		
	2days	3days	5days	2days	3days	5days	2days	3days	5days
Sodium bisulphite (0.1%)	CL	CL	CL	NC	NC	NC	99.99	99.91	99.16
Sodium sulphite (0.1%)	CL	CL	CL	NC	NC	T	98.91	98.47	98.07
Sodium metabisulphite (0.2%)	CL	CL	CL	NC	T	T	91.69	85.23	82.12
Ascorbic acid (0.1%)	R	R	R	NC	NC	NC	88.01	74.76	69.78

CL - Colourless, NC - No change, T - Turbidity, R - Red

The results of the study showed that the sodium bisulphite (0.1%) was the most suitable antioxidant for the formulation of the product.

Composition of aqueous injection

Table 15: Composition of aqueous injection formulation of nifedipine.

S. no.	Product code	Ingredients	Prescribed formula	Working formula
1.	$IBUCA_3$	Nifedipine	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

pH of aqueous injection: The pH of the developed formulation was found to be **7.43**.

Stability monitoring: The prepared formulation was unaffected in respect of colour stability. No visual colour change or precipitate was revealed in the developed formulation.

Table 16: Physical stability data for nifedipine injection IBUCA$_3$.

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

Freeze thaw cycle: There was no precipitation and no turbidity in the developed parenteral formulation at the end of the testing.

IN-Vitro evaluation

Table 17: Precipitation of nifedipine 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	-	-	-	-	-	-	-	-	-	-	-	-	-	-

No precipitation was observed on dilution in the developed formulation.

SUMMARY AND CONCLUSION

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 of a poorly water-soluble drug. In the present study, slightly water-soluble drug, nifedipine

was tried to solubilize by employing the combination of physiologically compatible hydrotropic agents to attempt its parenteral and oral liquid formulations.

The melting point determination and spectophotometric analysis of the drug sample were carried out as per the requirements of Indian Pharmacopoeia 1996. The drug complied with the tests prescribed in the monograph. The Infrared spectra of the dug showed major peaks at wavenumbers that are characteristic of nifedipine. The calibration curve of the drug was prepared in the water for solubility analysis. The linearity of calibration curve showed that the Beer Lamberts law was obeyed in the concentration range of 5-25 µg/ml at the λ_{max} of 328 nm. Preformulation study of the drug was carried out to determine the solubility of drug in water. Aqueous solubility of drug was found to be 0.0108% w/v.

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 determination of drug in hydrotropic solutions was carried out at room temperature. The solubility was increased upto 197.46 fold in 40% sodium benzoate solution, 14.44 fold in 40% urea solution, 10.85 fold in 40% sodium citrate solution and 9.06 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 nifedipine 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. An extremely high synergistic effect of hydrotropic agents was observed in the blends $BUCA_2$ and $BUCA_3$ containing all four agents in ratio of 20:10:5:5 and 10:20:5:5 respectively. This shows that mixed hydrotropy plays significant role in improving the solubility of practically insoluble drug, in the present study, furosemide, by employing the combination of agents at lower concentration. To check the influence of pH on the solubility of nifedipine, buffer solution of pH 8.0, 8.4 and 9.0 were used. 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. 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) of nifedipine. 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 prepared formulations were subjected to physical stability testing 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. 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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