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Hydrotropic solubilization of nimesulide for parenteral administration

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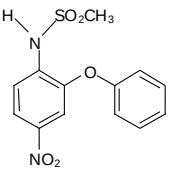
Abstract

Nimesulide is a non-steroidal anti-inflammatory drug (NSAID) that exhibits analgesic, antipyretic and anti-inflammatory activities. It is practically insoluble in water. The effect of various hydrotropes such as nicotinamide, sodium ascorbate, sodium benzoate, sodium salicylate and piperazine on the solubility of nimesulide was investigated. The solubility enhancement of nimesulide by the hydrotropes was observed in decreasing order as piperazine > sodium ascorbate > sodium salicylate > sodium benzoate > nicotinamide. In order to elucidate the probable mechanism of solubilization, various solution properties of hydrotropes such as viscosity, specific gravity, surface tension, refractive index, specific conductance of hydrotropic solutions were studied at 25 ± 2 °C on the basis of earlier studies. The hydrotropic solubilization of nimesulide at lower hydrotrope concentration may be attributed to weak ionic interactions while that at higher hydrotrope concentration may be due to molecular aggregation. Parenteral formulations using piperazine as a hydrotrope were developed and studied for physical and chemical stability.

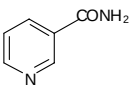
Keywords: Hydrotropic solubilization; Nimesulide; Parenteral formulation; Piperazine

1. Introduction

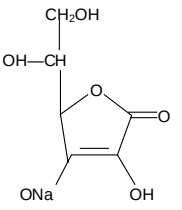
Nimesulide is an acidic non-steroidal anti-inflammatory agent, which differs from many similar compounds in that it is acidic by virtue of a sulfonanilide rather than a carboxyl group. It is an inhibitor of cyclo-oxygenase 2, hence inhibits the synthesis of destructive prostaglandins and spares cytoprotective prostaglandins. Nimesulide is practically insoluble in water (0.01 mg/ml), which precludes its use in parenteral formulations. The chemical structure of drug and various hydrotropes used in this study are shown below:



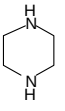
Nimesulide



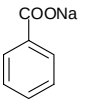
Nicotinamide



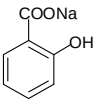
Sodium ascorbate



Piperazine



Sodium benzoate



Sodium salicylate

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The purpose of the present study was to investigate the effect of hydrotropic solubilization on the solubility of nimesulide, and to attempt formulation in aqueous solutions for parenteral use (Woolfson et al., 1986; Etman and Nada, 1999; Li et al., 1999a,b; Jain et al., 2001). Formulations were also studied for physical and chemical stability.

2. Materials and methods

2.1. Materials

The gift sample of nimesulide was provided by M/S Alkem Laboratory, Mumbai, India. Piperazine was procured from Fluka Chemicals, Germany; sodium ascorbate, sodium benzoate, and sodium salicylate from Loba Chemie, Mumbai, India.

2.2. Estimation of nimesulide

In the present study, UV spectrophotometric method (Piel et al., 1997) was used for the estimation of nimesulide. The calibration curve of nimesulide was prepared using 0.1N sodium hydroxide at 393 nm using double-beam spectrophotometer (UV-1601, Shimadzu, Japan).

2.3. Solubility study

An excess quantity of nimesulide was added to screw capped 30 ml glass vials containing the different aqueous systems viz. distilled water, buffers of pH 2.5–10 and hydrotropic solutions of different concentrations (0.4–2.0 M) in water. The vials were shaken mechanically for 12 h at 25 ± 2 °C and 37 ± 2 °C in a mechanical shaker (Elico Pvt. Ltd, Mumbai, India). These solutions were allowed to equilibrate for next 24 h and then centrifuged for 5 min at 2000 rpm. The supernatant of each vial was filtered through Whatman filter paper no. 1, filtrate diluted with suitable quantity of 0.1N NaOH and analyzed spectrophotometrically at 393 nm. The solubility of nimesulide was determined in triplicate.

2.4. Properties of hydrotropic solutions

In order to interpret the probable mechanism of solubilization, UV spectral studies of nimesulide were performed in different hydrotropic solutions to study the possible spectroscopic changes in the structure of nimesulide in presence of different hydrotropes. The various solution properties of hydrotropes such as pH, viscosity, specific gravity, surface tension, refractive index, conductance and diffusion rate were also studied in an attempt to reason out the increase in solubility of nimesulide with increase in hydrotrope concentration (Saleh et al., 1983; Saleh and El-Khordagui, 1985).

2.5. Formulation of aqueous injection

On the basis of solubility data obtained, two formulations of aqueous injection of nimesulide NPZ_1 and NPZ_2 were prepared using piperazine as hydrotrope (Sweetana and Akers, 1996; Nema et al., 1997). The formulation NPZ_1 contained 10 mg/ml of nimesulide in 1.6 M piperazine solution and formulation NPZ_2 contained 33.3 mg/ml of nimesulide in 2 M piperazine solution. In both the formulations, 0.1% w/v sodium bisulfite was added as an antioxidant. Other additives like chelating agent and buffering agent were not included in these formulations as they might lead to change in the solubility behavior and upset the basic solubility enhancement ratio.

For the preparation of aqueous injection of nimesulide, 25 ml piperazine solution was placed in 30 ml screw capped glass bottles and weighed amount of nimesulide and 0.1% w/v sodium bisulfite were added to the bottles. The contents of these bottles were shaken for 12 h on a mechanical shaker for complete solubilization and equilibrated for additional 12 h. These solutions were filtered through Whatman filter paper no. 1 and then through 0.45 μm membrane filter (Sartoroius, Germany). The solutions were analyzed spectrophotometrically at 393 nm for drug content after appropriate dilutions with 0.1N NaOH.

2.5.1. Treatment of packing material

Clear glass vials of 3 and 5 ml capacity were first washed several times with distilled water. All these vials were dried and sterilized by dry heat in an oven at 160 °C for 2 h in inverted position. Rubber stoppers used for plugging the vials were first washed several times with distilled water and then autoclaved at 15 lbs pressure (120 °C) for 20 min and finally dried in vacuum oven.

2.5.2. Preparation of aseptic area

The walls and floor of aseptic room were thoroughly washed with water and then disinfected with 5% w/v phenol solution. The laminar airflow bench was cleaned with 70% v/v ethanol and the UV light was switched on for 30 min prior to filling of injections into vials.

2.5.3. Aseptic filtration

The aqueous solutions of nimesulide were prepared as above and sterilized by filtration through 0.2 μm disposable membrane filter (Sartorius, Germany), fitted in a holder of 5 ml glass syringe and the pressure on the piston was adjusted.

2.5.4. Final flushing with nitrogen gas

The sterile aqueous solution of nimesulide was flushed with sterile nitrogen gas aseptically and 3 and 5 ml volumes were filled into vials.

2.6. Stability studies

2.6.1. Physical stability studies

The sealed vials of the formulations were visually inspected every day for 30 days against black and white backgrounds to see the changes occurring, if any, in physical appearance of aqueous injection like color, turbidity, precipitation etc., on storage at 25 ± 2 °C (RT), 4 ± 2 °C in a refrigerator, 45% relative humidity (RH). For freeze thaw cycling the vials were kept alternately at 45 ± 2 and 4 ± 2 °C for 24 h and shaken every day for 10 min on a mechanical shaker.

2.6.2. Chemical stability studies

The formulations were subjected to exhaustive chemical stability at 25 ± 2 °C, 37 ± 2 °C, and 45 ± 2 °C in thermostatically controlled ovens for a period of 4 weeks. The formulations were analyzed spectrophotometrically initially and at intervals of seven days upto 4 weeks to calculate the drug content. The percent residual drug for each formulation at different time intervals as well as at different temperatures was calculated considering the initial drug content for each formulation to be 100%. From the Arrhenius plots, the K values at 25 °C were determined by extrapolating the graph. The time period required for 10% degradation of drug ($t_{10\%}$) for each formulation was calculated.

3. Results and discussion

The results of solubility studies at different pH indicated that nimesulide was more soluble at alkaline pH than acidic pH. This may be due to the acidic nature of nimesulide by virtue of its sulphonanilide group. The aqueous solubility of nimesulide was increased upto 79 times at pH 10.0 (Table 1).

The solubility of nimesulide was found to increase upto 3248 times at 25 ± 2 °C in 2 M piperazine solution. It is evident from the solubility data of nimesulide that by increasing the temperature from 25 ± 2 °C to 37 ± 2 °C, the solubility of drug was increased; showing that solubilization of nimesulide was endothermic. The solubility enhancement power of different hydrotropes could be ranked in decreasing order as—piperazine > sodium ascorbate > sodium salicylate > sodium benzoate > nicotinamide as shown in Figs. 1 and 2 and the solubility enhancement ratio for 2 M hydrotrope concentration was found to be 3248 > 156 > 68 > 58 >12, respectively (Table 2).

The increase in solubility is not the linear function of the hydrotrope concentration. On increasing the hydrotrope concentration, initially the drug solubility was increased slowly but after a particular concentration, i.e. critical solute concentration (CSC) of

Table 1
pH-dependent solubility (mg/ml) of nimesulide at different temperatures

	Solubility[a] (mg/ml) in PBS of different pH							
	2.5	4.0	6.8	7.0	7.5	8.0	9.0	10.0
25 ± 2 °C	0.0258	0.0281	0.0299	0.0364	0.0386	0.0482	0.0853	0.0892
37 ± 2 °C	0.0268	0.0297	0.0312	0.0380	0.0392	0.0484	0.0921	0.0992

[a] Average of three observations.

Table 2
Solubility enhancement ratio of nimesulide in hydrotropic solutions at 25 ± 2 °C

Hydrotrope	Solubility enhancement ratio[a] of nimesulide in different molar concentrations of hydrotropes				
	0.4 M	0.8 M	1.2 M	1.6 M	2.0 M
Nicotinamide	2.70	4.48	7.75	9.98	11.99
Sodium ascorbate	4.14	61.67	65.23	88.33	156.59
Piperazine	88.70	187.64	292.85	566.142	3248.48
Sodium benzoate	6.33	8.94	9.26	15.60	58.37
Sodium salicylate	20.25	24.01	28.94	52.63	67.72

[a] Average of three observations.

the hydrotrope, there was a tremendous increase in the solubility of nimesulide.

The higher solubility of nimesulide in presence of one hydrotrope over other can be explained on the basis of Poochikian and Gradock's (1974) explanation. The hydrotropes selected for the present study (nicotinamide, sodium benzoate and sodium salicylate) possess a hydrophobic centre having the parent benzene nucleus which can interact due to a large surface area and a mobile electron cloud known as an aromatic sextet. Thus these sites are available for non-bonded and vander Wall's interaction with water and nimesulide. The molecules of water join to form cluster together. For solubilization the ionized hydrotropes break this association and use the ion dipoles of water for solvation. The increasing hydrotrope concentration results in unassociated form of water to make cluster of the hydrotrope by hydrogen bonding and non-bonding interactions at the various centers of drug molecule. Thus charge delocalization along with an increase in π-cloud area on hydrotropic molecule would account partially for difference in apparent drug solubility in presence of various hydrotropes (Poochikian and Gradock, 1974).

The UV absorption spectra of nimesulide in various hydrotrope solutions showed a slight shift in λ_{max} (393 ± 1 nm), which can be due to minor electronic changes in the structure of drug molecules. There is no base to assume that there was any complex formed between drug and hydrotrope molecules, because the complex formation can be evidenced by formation of new chromophores (by appearance of a new peak or merging of two peaks to generate a common peak).

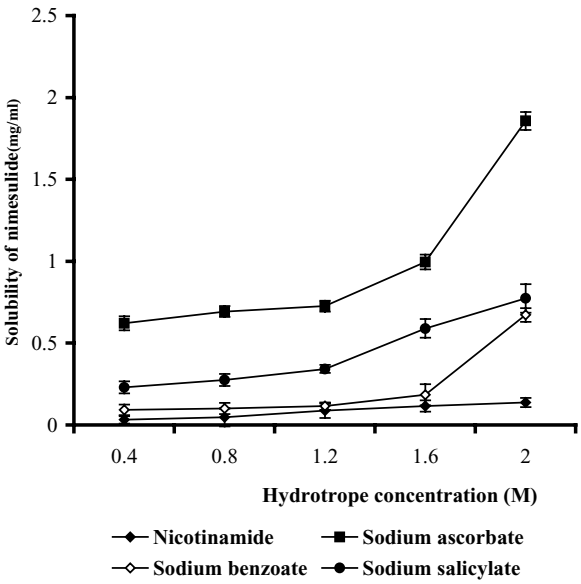

Fig. 1. Comparative equilibrium solubility of nimesulide in various hydrotropes.

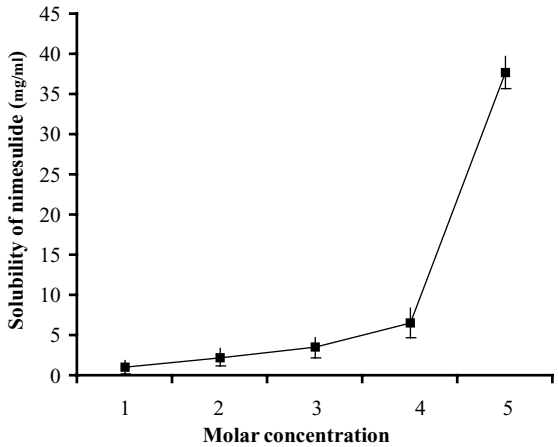

Fig. 2. Equilibrium solubility of nimesulide in piperazine.

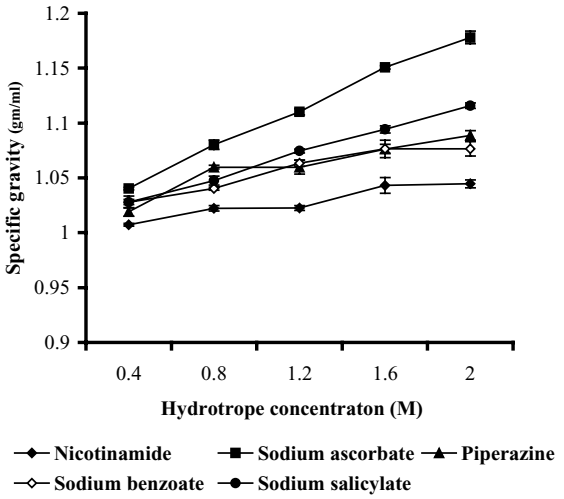

Fig. 3. Plots of specific gravity vs. molar hydrotrope concentration.

The plots of specific gravity versus hydrotrope concentration showed a negative deviation (Fig. 3) that indicates an increase in partial molal volume upon aggregation, and this increase in volume may be due to expansion of the hydrocarbon portion of the molecule or its partial removal from the high compressive force of water (Badwan and El-Khordagui, 1983). The positive deviation in the viscosity plots (Fig. 4) indicates that aggregate formation is associated with an

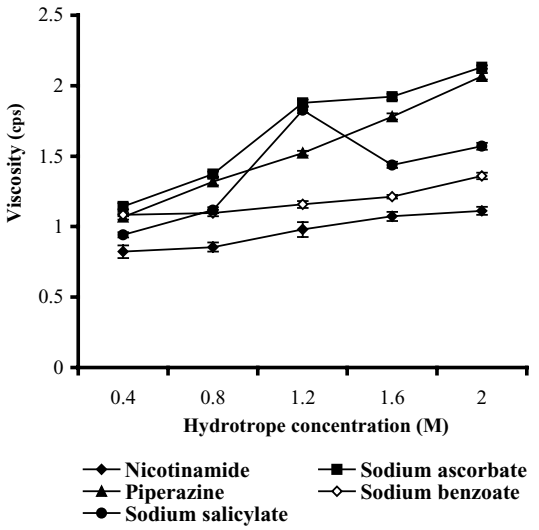

Fig. 4. Plots of viscosity vs. molar hydrotrope concentration.

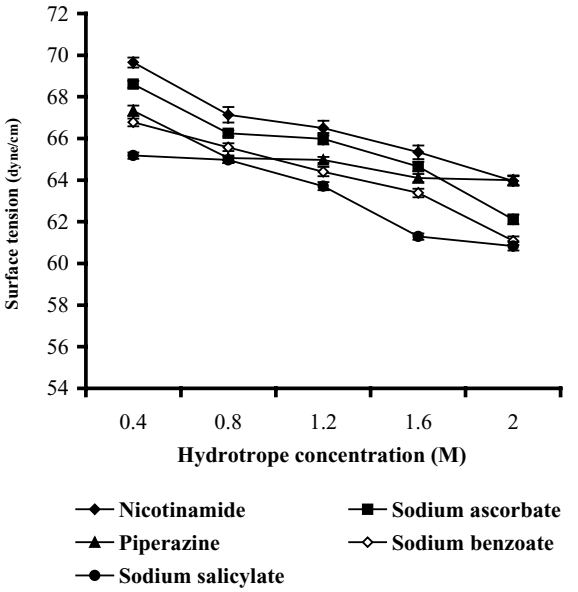

Fig. 5. Plots of surface tension vs. molar hydrotrope concentration.

increase in viscosity of hydrotrope concentration, which is in agreement with the self-association of phenolic compounds (Thomas, 1960). The surface tension plots (Fig. 5) showed a moderate decrease in surface tension on increasing the hydrotrope concentration as hydrotropes are not surface active agents (Saleh and Daabis, 1974; Saleh et al., 1983). The plots of refractive index versus hydrotrope concentration (Fig. 6) showed negative deviation. The deviation from linearity in specific conductance plots (Fig. 7) is strongly indicative of molecular aggregation (Mukerjee, 1967). It was revealed from different studies that at lower hydrotrope concentration, weak ionic interactions while at higher hydrotrope concentration, the molecular aggregation seems to be the possible mechanism of hydrotropic solubilization (Neuberg, 1916; Winsor, 1950; Saleh et al., 1983; Saleh and El-Khordagui, 1985; Yalkowsky, 1999; Ni et al., 2002).

The physical stability study showed that all formulations remain unchanged in respect of color stability and no turbidity or precipitate formation was observed at different storage conditions.

The data on chemical stability at different temperatures and time intervals are shown in Table 3 from which it can be inferred that the degradation of nimesulide follows first order kinetics. The calculated *K*

Table 3
Chemical stability data of formulations

Temperature (°C)	Formulation	Percent residual nimesulide[a] at time interval of				
		0 days	7 days	14 days	21 days	28 days
25 ± 2	NPZ_1	100.00	98.99	97.85	97.02	96.55
	NPZ_2	100.00	99.59	98.78	97.01	96.50
37 ± 2	NPZ_1	100.00	98.15	97.03	96.50	95.23
	NPZ_2	100.00	99.05	98.00	96.59	95.52
45 ± 2	NPZ_1	100.00	97.98	96.85	95.25	95.01
	NPZ_2	100.00	97.02	96.57	95.65	94.12

[a] Average of three observations.

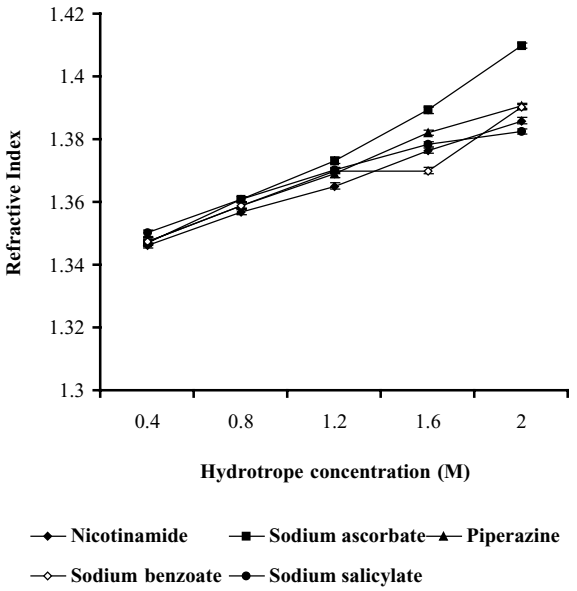

Fig. 6. Plots of refractive index vs. molar hydrotrope concentration.

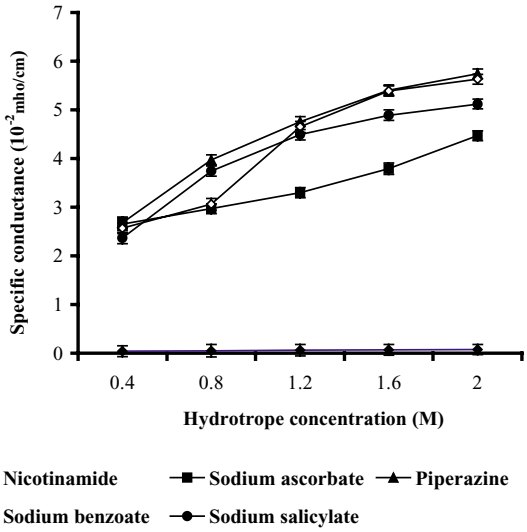

Fig. 7. Plots of specific conductance vs. molar hydrotrope concentration.

values, i.e. decomposition rate constant of formulations are reported in Table 4. The time required for the 10% degradation of drug for each formulation was calculated. The results show that the prepared formulations had a shelf life of 188 days necessitating the stabilization of prepared formulations by incorporation of appropriate additives.

Table 4
Degradation rate constant and shelf life of formulated products

Formulations	Degradation rate constant K ($days^{-1}$) × 10^{-3} at different temperatures (°C)			Shelf life (day)
	37 ± 2	45 ± 2	25 ± 2	
NPZ_1	7.210	9.75	5.714	182
NPZ_2	7.142	12.4	5.535	188

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