

Studies on Suspension of Nimesulide Solid Dispersion: Development, Characterization and *In vivo* evaluation

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The present study aimed at enhancing solubility and dissolution of nimesulide by applying solid dispersion technique followed by formulating it as suspension. Solid dispersions of nimesulide were prepared using propylene glycol, polyvinyl pyrrolidone-K30 and polyethylene glycol-6000 and formulated as respective suspensions. The suspensions were characterized by studying the particle size and sedimentation volume (Hu/Ho). *In vitro* evaluation was carried out in USP XXI dissolution apparatus. The *in vivo* evaluation of nimesulide suspensions were carried out in carrageenan induced rat paw edema method. Long term stability studies were carried out at different temperatures. Nimesulide suspensions with solid dispersion exhibited good suspendability and gave higher dissolution rates than those with plain nimesulide suspension. They also demonstrated enhanced anti-inflammatory activity when compared with plain drug suspension. The percentage edema inhibition obtained for different suspensions of nimesulide solid dispersion F2, F3 and F4 after 1 h were 76%, 79% and 82%, respectively. On the other hand edema inhibition of plain nimesulide suspension was only 40%. It can be concluded from the study that the suspension of nimesulide solid dispersion exhibited improved dissolution profiles, stability and *in vivo* efficacy.

Nimesulide, a methyl sulfonamide derivative, is relatively a new non-steroidal antiinflammatory, analgesic drug. It is a potent and selective cyclooxygenase-2 inhibitor, highly effective in the treatment of various forms of pain and inflammatory conditions with minimum drug related side effects¹. Because of its poor solubility (0.01 g/l in water)², nimesulide was selected as candidate for the present study on solid dispersion. The two most commonly used approaches for improving bioavailability are the particle size reduction and solubility improvement through systematic formulation approaches. The solid dispersion technique produces size reduction through the dispersion of drug in water soluble matrices either molecularly or as fine particles which increase the bioavailability of poor water soluble drugs³⁻⁶. The drug in a solid dispersion might not be in micro-crystalline state, but a certain fraction of the drug might be present in a molecularly dispersed state in the carrier matrix⁷. In the present study, a combination of sus-

pension and solid dispersion technique was tried. As both techniques involve size reduction but through different mechanism, combination of these techniques may give a leap in dissolution profiles. Polyvinyl pyrrolidone K30, PEG-400 and PG were tried as dissolution enhancers in the previously optimized surfactant system of Tween 80 for the preparation of nimesulide solid dispersion. The solid dispersions were used to formulate the suspensions. The physical stability and dissolution parameters of formulations were compared with that of pure suspension. *In vivo* study was carried out in rats by comparing the percent inhibition of carrageenan induced paw edema.

MATERIALS AND METHODS

Nimesulide was obtained as a gift sample from Dr. Reddy's Laboratories, Hyderabad. Carrageenan was obtained from Sigma, St. Louis, USA. The polymers and other chemicals such as Propylene glycol (PG), methyl cellulose (40 cps), polyvinyl pyrrolidone (PVP) K-30, PEG 6000, glycerin, sucrose, methanol, acetonitrile, diammonium phos-

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phate and sodium benzoate were procured from S. D. Fine Chem. Ltd., Mumbai. All other chemicals used were of analytical grade.

Estimation of nimesulide:

The analytical column used was NovaPak® C 18, 3.9×150 mm, (Waters Corporation). A combination of acetonitrile-diammonium phosphate buffer (0.01 M) in a ratio of 40:60 was used as a mobile phase at a flow rate of 1ml/min. The samples were analyzed with a UV detector set at 230 nm. The retention time of nimesulide was 1.9 to 2.1 min. The HPLC system was interfaced with Microsoft Windows® 95 was run by Breeze software.

Preparation of nimesulide solid dispersion:

Solid dispersions of nimesulide in PG (or) PVP (or) PEG-6000 were prepared by conventional solvent evaporation method. The drug and carrier were dissolved in minimum volume of methanol and the solvent was removed by using vacuum evaporator. The solid dispersion was kept under vacuum till the solvent was completely removed and was confirmed by GC analysis. Solid dispersions were then pulverized in mortar and pestle, passed through a mesh size 40, and then stored in a desiccator.

Preparation of nimesulide suspension:

Five milliliters of suspensions containing 50 mg nimesulide were prepared as per formulae given in Table 1. Accurately weighed quantity of nimesulide or solid dispersion was taken in mortar and lavigated with a small portion of methylcellulose mucilage. When a smooth paste formed the rest of the methylcellulose mucilage was added in a divided portion while triturating the contents. Sucrose was then added as a solution in water while mixing. Other ingredients were added one after another, mixed and the suspension was transferred to a measuring jar and adjusted the volume.

Particle size measurement and sedimentation study:

Size of the nimesulide particles in the suspension was measured by microscopy. Average particle size and standard deviation of 100 particles was estimated. The results are given in Table 2. In sedimentation study, the suspensions were transferred to a stoppered measuring cylinder and were stored at room temperature (27±1°). The volume of sediment formed was noted at regular intervals of time. The sedimentation volume, ratio of the ultimate height (Hu) of the sediment to the initial height (Ho) of the suspension (i.e. Hu/Ho)⁸ were calculated and the results are given in

the Table 2.

TABLE 1: FORMULAE OF NIMESULIDE SUSPENSIONS.

Ingredients (g)	Formulations			
	F ₁	F ₂	F ₃	F ₄
Nimesulide	1.00	-	-	-
Nim-PG(4:1)	-	1.25	-	-
Nim-PVP(4:1)	-	-	1.25	-
Nim-PEG 6000(4:1)	-	-	-	1.25
Methylcellulose	3.0	3.0	3.0	3.0
Glycerin	15	15	15	15
Sucrose	25	25	25	25
Sod. Benzoate	0.2	0.2	0.2	0.2
Colour	q.s.	q.s.	q.s.	q.s.
Purified water (ml) to	100	100	100	100

Suspensions formulated employing nimesulide alone (F₁) and its solid dispersion in PG (F₂), PVP-k30 (F₃) and PEG-6000 (F₄)

TABLE 2: STABILITY AND DISSOLUTION PARAMETER OF NIMESULIDE SUSPENSIONS.

Formulations	pH	Particle size(μ) (X±SD)	Sedimentation volume (Hu/Ho)	DE ₃₀ % (X±SD)	T ₅₀ (Min)
F ₁	6.21	15.2±8.40	0.99	48.8±4.60	32
F ₂	6.11	9.2±5.40	1.00	69.8±6.84	14.8
F ₃	6.15	8.84±5.10	1.00	70.2±6.00	12
F ₄	6.08	8.02±5.60	0.98	74.2±5.10	10.2

DE₃₀% is percentage dissolution efficiency of 30 min and T₅₀ is time for 50% release

Dissolution rate study:

The dissolution rate of nimesulide from various suspensions was studied using USP XXI dissolution apparatus employing a paddle stirrer. In 900 ml of alkaline borate buffer (pH 7.4), a sample of suspension equivalent to 50 mg of nimesulide a speed of 50 rpm and a temperature maintained at 37±1° were employed in each test. A 5 ml aliquots of dissolution medium collected at different inter-

vals of time (0, 10, 20, 30, 45, 60, 90 and 120 min) were filtered through 0.45 μm syringe filters and analyzed. The dissolution experiments were conducted in triplicate.

Stability studies:

The suspensions were placed in screw capped glass containers and stored at ambient humidity conditions, at room temperature ($27\pm 2^\circ$), oven temperature ($40\pm 2^\circ$) and in refrigerator (5° – 8°) for a period of 26 weeks. The suspensions were examined for potency, crystallization and dissolution efficiency at regular intervals.

Antiinflammatory activity:

Screening for antiinflammatory activity was carried out by carrageenan-induced paw edema method in male Wistar rats. The experimental protocol was approved by Institutional Animal Ethics Committee, Jamia Hamdard, New Delhi. The animals were divided into five groups of 6 animals each. Group one received the suspension without drug and served as control. Group two received nimesulide pure drug suspension (5 mg/kg) and served as standard. Group three received treatment with nimesulide-PG suspension. Group four received treatment with nimesulide-PVP-K30 suspension. Group five received treatment with nimesulide-PEG 6000 suspension. After 30 min 0.1 ml of 1% carrageenan in saline was injected by sub plantar route into the right hind paw. The volume of paw was measured immediately with help of a plethysmometer. This reading was assigned as zero hour volume. The volume of the right paw was again measured subsequently at 0.5, 1.0, 1.5, 2, 2.5, 3.0 and 4.0 h. The swelling in the animals treated with drug was compared with that of control and the percentage inhibition of edema was calculated using the formula⁹. Percent reduction of edema = $(C - T/C) \times 100$, where C is mean volume of edema for control and T is mean volume of edema for treated group.

RESULTS AND DISCUSSION

In this study solid dispersion of nimesulide was formulated into nimesulide suspensions. Suspension F_1 was formulated without solid dispersion of nimesulide, whereas suspensions F_2 , F_3 and F_4 were formulated employing solid dispersion of nimesulide in PG, PVP, and PEG-6000. The average particle size in F_1 was found to be 15.2μ whereas the average size in suspension F_2 , F_3 and F_4 was found to be 9.2, 8.84 and 8.02μ , respectively. The particle size (Table 2) was very much less in the suspensions formulated employing solid dispersion. This is very much in accordance with the earlier study on suspensions of ibuprofen solid

dispersions¹⁰. Sedimentation values (F) determined for all the suspensions were 1 (or) less. All the suspensions formulated exhibit good suspendability of nimesulide. No sedimentation occurred in the prepared suspensions when observed for 26 weeks. The suspensions formulated were very uniform without any flocculation. The dissolution profile of nimesulide from various suspensions are summarized in (Table 2) and shown in (fig.1). Dissolution efficiency DE30 (%) values were calculated as suggested by Khan¹¹. Dissolution efficiency DE30 (%) of the various formulations were found to be F_1 (49), F_2 (70), F_3 (70) and F_4 (74). Suspensions formulated employing solid dispersion F_2 , F_3 and F_4 gave significantly ($p < 0.05$) higher dissolution rate than formulation F_1 . The enhanced dissolution rate in the suspensions with the solid dispersion can be attributed to their smaller particle size when compared to other suspension.

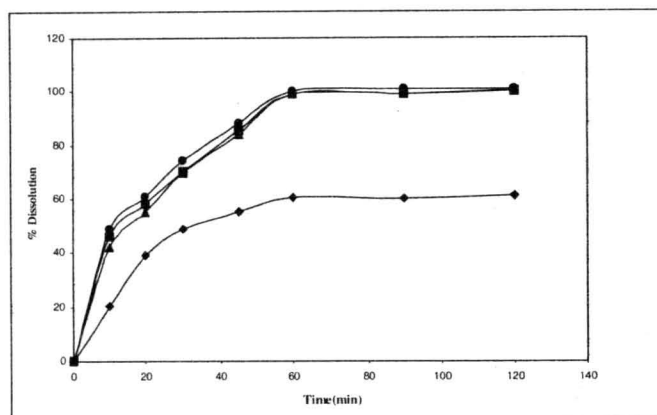


Fig. 1: Dissolution profile of nimesulide suspensions.
Dissolution profiles of nimesulide suspensions formulated employing nimesulide alone (♦), and its solid dispersion in PG (▲), PVP-K30 (■) and PEG-6000 (●).

The solid dispersion using PEG-6000, PVP and PG in the suspension exhibited the higher dissolution rate. This can be attributed to several factors. The polymer PEG-6000 is crystalline and water soluble with two parallel helices in a unit cell. Significant amount of drug can be trapped in the helical interstitial space when PEG-6000 and drug are solidified. It thus acts to reduce the size of the drug particle by decreasing their aggregation and agglomeration and thereby contributing to the enhancement of dissolution of the drug. While the polymer PVP-K30 enhances the solubility by retarding the crystallization of poorly water-soluble drugs. It may also contribute to the solubility enhancement by improving the wettability of the drug particles due to its

surfactant property. On the other hand the propylene glycol is an efficient solubilizer, and thereby contributing to the increased dissolution of the drug. Thus the improvement of dissolution profile may be expected to give enhanced bioavailability.

The formulations involving solid dispersion showed excellent stability with respect to physical changes, potency and dissolution efficiency (Table 3). They were monitored for formation of crystal for 26 w. There were no occurrences of crystallization in any of the suspensions during the period.

The carrageenan induced rat paw edema method was used as a tool to compare efficacy of the solid dispersion suspensions with the plain drug suspension. The results were expressed as percent edema inhibition and are shown graphically (fig. 2). The percent edema inhibition of the suspension F₁, F₂, F₃ and F₄ were shown to be 40%, 76%, 79% and 82% at 1 h, respectively. The results reveal that efficacy of suspensions by solid dispersion technique is higher than that of plain drug suspension. The data were compared by ANOVA technique and p<0.05 was considered as significant.

The simplicity of method and ease of scale up for bulk

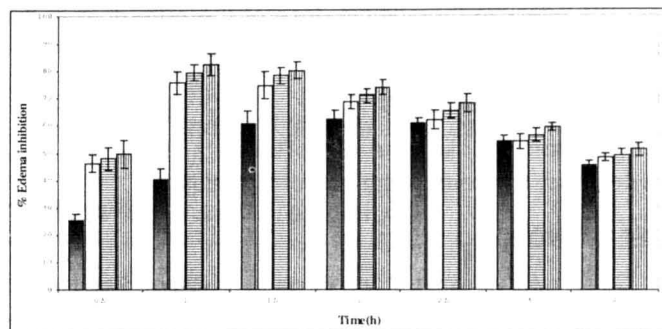


Fig. 2: Histogram of antiinflammatory efficiency of nimesulide suspensions.

Anti-inflammatory effect of nimesulide suspensions formulated employing nimesulide alone (■) and its solid dispersion in PG (□), PVP-K30 (▨) and PEG-6000 (▩).

manufacturing renders nimesulide suspension with solid dispersion an economically viable preposition for enhancing the therapeutic efficacy of nimesulide from their oral dosage forms. Suspensions with solid dispersion of nimesulide:PG (4:1), nimesulide:PVP (4:1), nimesulide:PEG 6000 (4:1) exhibited high dissolution rates and stability. DE₃₀% of their formulations was higher than of pure drug suspension and also the formulations provided quicker

TABLE 3: STABILITY STUDIES OF SOLID DISPERSION OF NIMESULIDE SUSPENSIONS UNDER ACCELERATED STORAGE CONDITION.

Time point	Storage	Formulation F2			Formulation F3			Formulation F4		
		Potency (%) ^a	crystal ^b	DE ₃₀ (%) ^c	Potency (%) ^a	crystal ^b	DE ₃₀ (%) ^c	Potency (%) ^a	crystal ^b	DE ₃₀ (%) ^c
Initial		99.2	Nil	69	99.4	Nil	70	99.1	Nil	74
2 w	5°	-	-	-	-	-	-	-	-	-
	25°/60% RH	99.2	Nil	68	99.0	Nil	70	99.2	Nil	74
	40°/75% RH	98.2	Nil	67	99.2	Nil	68	99.4	Nil	72
4 w	5°	97.8	Nil	67	96.8	Nil	69	96.5	Nil	72
	25°/60% RH	98.0	Nil	68	98.2	Nil	70	98.0	Nil	74
	40°/75% RH	97.2	Nil	66	96.5	Nil	68	98.6	Nil	73
13 w	5°	96.8	Nil	67	97.3	Nil	70	97.2	Nil	73
	25°/60% RH	98.3	Nil	67	95.8	Nil	69	96.8	Nil	71
	40°/75% RH	97.4	Nil	65	95.7	Nil	67	97.6	Nil	68
26 w	5°	95.2	Nil	68	97.3	Nil	68	95.7	Nil	70
	25°/60% RH	96.8	Nil	66	96.8	Nil	69	96.2	Nil	72
	40°/75% RH	96.2	Nil	63	97.5	Nil	65	95.2	Nil	70

^apotency determined using HPLC assay described in the experimental section, ^b presence of crystals determined by microscopic analysis and ^cpercentage dissolution efficiency of 30min

onset of action and high efficiency in antiinflammatory studies. The effective therapeutic dose of the pure drug in the formulation can be decreased accordingly.

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