Design And In Vitro Evaluation of Nimesulide Loaded Solid Lipid Nanoparticles (SLN's)

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Abstract :

Purpose : The purpose of present investigation was to prepare solid lipid nanoparticles of Nimesulide (NSL) and to evaluate and characterize the prepared optimum formulation.

Methods : This technique was selected on the basis of production yield, ease of formulation and practicability. For improving oral bioavailability, Nimesulide loaded SLNs were developed using glyceryl monostearate was the best lipid for NSL SLNs. Polyvinyl alcohol was used as surfactant. SLNs were characterized for particle size, entrapment efficiency, drug loading, In-vitro drug release measurements for the analysis of polymorphic modifications.

Results : Particle size of SLNs was measured by Malvern Zetasizer and under Projection Microscope and was found to be in the limit of 300-500 nm and also had almost round and uniform shape. *In-vitro* release studies showed that Nimesulide (NSL) was released from SLNs in a sustained manner for all formulations.

Conclusions : Thus, it can be concluded that a systematic formulation approach can be adopted to reach an optimum point in the shortest time with minimum efforts.

Keywords : Nanotechnology, Lipids, Surfactants, entrapment efficiency, drug release.

Introduction[1, 2]:-

Nanotechnology is a field of applied science and technology wherein the particles smaller than 1 µm, normally 50-1000 nm are obtained which also includes the development of devices within that size range. This field includes the applications from various scientific disciplines such as materials science, applied physics, colloidal science, pharmaceutical sciences, supramolecular chemistry, electrical engineering, device physics and even mechanical engineering. In this technology the drug is not only dissolved and entrapped, but also attached to a nanoparticle matrix or encapsulated. Solid Lipid nanoparticles (SLN) system includes the surrounding of the drug by a lipid membrane by confining it into a cavity. Depending upon the method of preparation, solid lipid nanoparticles can be obtained.

Solid lipid nanoparticles (SLN) have emerged as one of the Novel drug delivery systems that can provide controlled drug delivery for a long duration of time. SLNs are particles of nanometer range prepared using solid lipid and stabilized by surfactants, sometimes referred as lipospheres or nanospheres. SLNs are novel development in the field of nanotechnology and are emerging as a promising strategy for the efficient delivery of poorly water soluble drugs. In the present research study Nimesulide SLNs have been developed by solvent diffusion method. It is an alternative drug delivering system to traditional colloidal system like emulsions, liposomes and polymeric particles. Nimesulide is an NSAID's drug with good lipid solubility, poor oral bioavailability (near about 65%) which may be attributed to its poor water solubility and the first pass metabolism.

For improving oral bioavailability, Nimesulide loaded SLNs were developed using Glyceryl Behenate, Glyceryl monostearate and stearic acid. PVA, Span 80 and Tween 80 or were used as surfactants. Solvent Diffusion Method was selected to prepare SLN dispersions. SLN were characterized for Particle Size, entrapment efficiency, Drug loading and in vitro drug release measurements for the analysis of polymorphic modifications. Projection microscope for particle morphology as well as for particle size analysis was used.

Particle size of SLN was measured by Malvern zetasizer and was obtained in the range of 300-500 nm. In vitro release studies were performed by dissolution for 12 hrs which shows sustained and prolonged release kinetics. This novel approach has immense potential for commercialization.

Solid lipid Nanoparticles are made from solid lipids are attracting. The solid lipid nanoparticles are submicron colloidal carriers (50-1000nm) which are composed of physiological lipids, dispersed in water or in an aqueous surfactant solution. Nimesulide is an NSAID's drug having high lipophilicity compound with half life of about 3hr. Approximate 50% orally administered dose is absorbed but absolute bioavailabily is only about 65% due to first pass metabolism. Selection of surfactant is important because it prevents globule coalescence during diffusion step.[1]

Experimental :

Preformulation :

1) Materials : Nimesulide was obtained as a gift sample Emcure Pharmaceuticals, Pune (M.S). Glyceryl Monostearate, Glyceryl Behenate, and Stearic acid were obtained from Lobachemie, Mumbai. The organic solvents and other reagents of analytical grade were used. Double-distilled water was used for the preparation of buffer and its further dilutions.

2) Drug Profile[2] :- Nimesulide is an NSAID's drug having high lipophilicity compound with half life of about 3 hr. Approximate 50% orally administered dose is absorbed but absolute bioavailabily is only about 65% due to first pass metabolism.

3) Table no.1 Lipid profile :-[2-3]

Sr. No	Name	Synonyme	Melting Point	Solubility	Insolubility
1	Glyceryl Monostearate	Imwitor	≥ 55◦c	ethanol, Ether, Chloroform,	Practicaly in Water
2	Glyceryl Behenate	Compritol 188 ATO	65-77◦c	Chloroform, Dicloro-methane	Practicaly in Ethanol Hexane, Water.
3	Stearic acid	Edenor	≥ 54◦c	Ethanol Hexane, Propylene Benzene,	Practicaly in Water

4) Calibration Curve :-

Calibration curve of Nimesulide was obtained using an UV-Spectrophotometer (UV 530 JASCO) in both the mediums i.e. 0.01N HCl and distilled water at 450 nm wavelength.

Preparation of 50 µg/ml Stock Solution :-

Stock solution of Nimesulide was obtained by dissolving 5mg Nimesulide in sufficient amount of 0.01N HCl in a volumetric flask (100ml). The solution was sonicated for 5 min followed by the final volume adjustment upto 100 ml with 0.01N HCL to get 50 µg/ml.

Table no.2 Dilution Factor :-

Sr. No	Pipette Out (ml)	Dilute with Medium (ml)	Total Concentration (µg/ml)
1	0.5	9.5	5
2	1	9	10
3	1.5	8.5	15
4	2	8	20
5	2.5	7.5	25
6	3	7	30

Observation Table :-

Table no. 3: Dilution for calibration curve D. W

Sr. No	Concentration (µg/ml)	Absorbance (nm)
1	0	0
2	5	0.1681
3	10	0.3511
4	15	0.5529
5	20	0.7019
6	25	0.9036

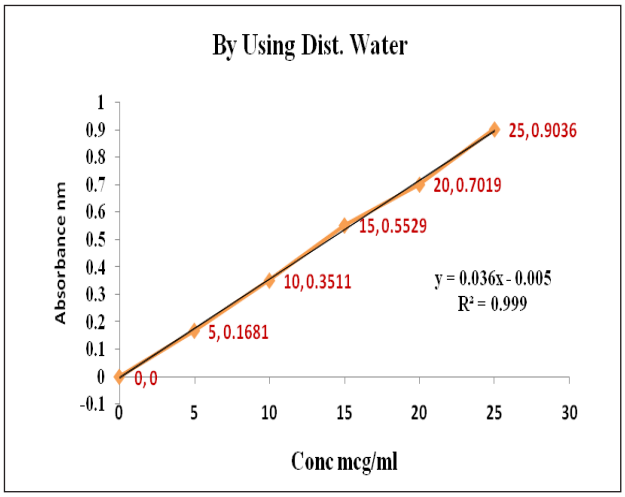
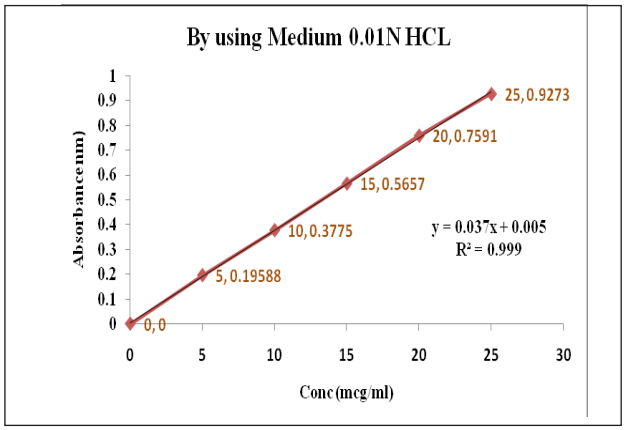


Fig. 1 Calibration curve for Dist. Water

Table no. 4: Dilution for Calibration curve 0.01 N HCL

Sr. No	Concentration (µg/ml)	Absorbance (nm)
1	0	0
2	5	0.19588
3	10	0.3775
4	15	0.5657
5	20	0.7591
6	25	0.9273

Fig. 2 Calibration curve for 0.01N HCL



5) Fourier transform-Infra red spectra : - IR is useful tools for investigating structural properties of lipid and drug. To ensure the compatibility of the Nimesulide with lipid preformulation studies were done using IR spectrum recorded on FT-IR (Perkin Elmer FT-IR system, spectrum BX) by preparing KBr disk.

Method of Preparation[4-5]:-

Principle :

Emulsification / solvent diffusion (ESD) was based on the use of organic solvents, the encapsulating lipid is made soluble in a partially water soluble solvent like acetone and ethanol mixture which was saturated using Distilled for obtaining the thermodynamic equilibrium. An organic solvent is not properly miscible with water so it becomes necessary that the solvent of the disperse phase should diffuse by dilution with excess water to obtain the precipitation of the lipid and the consequent formation of solid lipid nanoparticles. Subsequently, the lipid-water saturated solvent phase is emulsified in an aqueous solution containing stabilizer, leading to solvent diffusion to the external phase and the formation of solid lipid nanoparticle according to the oil-to-lipid ratio.

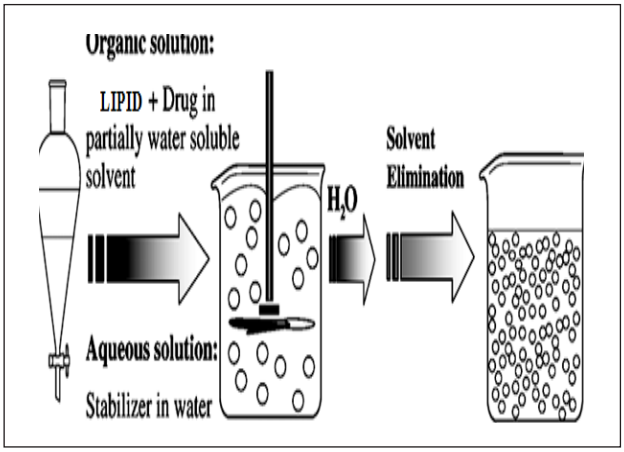


Fig. 3 Schematic illustration of the ESD technique.

Preparation of SLN [6-7]:-

The preparation of SLN was based on the principle of 'emulsion solvent diffusion method' in water, 150mg of Nimesulide and 150 mg glyceryl monostearate were put into a mixture of acetone/ethanol (15 ml each) and heated to 60–70 ^{0}C in a water bath. The total drug: lipid ratio was maintained at 1:1 W/W. The resulting solution was poured into 25 ml of 1% w/v aqueous PVA at 4–8 ^{0}C under mechanical stirring. The SLNs formed instantaneously, were recovered by centrifugation at 35,000 rpm for 30 min at 4–8 ^{0}C. The pellet was washed thrice with distilled water and vacuum dried.

This technique possesses various advantages like improved encapsulation efficiency (usually higher than 70%), simplicity, avoiding need of homogenization, narrow particle size distribution enhanced reproducibility batch-to-batch and easier scale-up.

2) Evaluation[7-8]:-

A) Particle Size Analysis :

Particle size of Nimesulide loaded SLNs were measured by photon correlation spectroscopy using Zetasizer (PCS3000, Malvern, English). Samples were diluted appropriately with aqueous solution containing 1% F68 and 20% sugar.

B) Entrapment Efficiency : -

The drug entrapment efficiency (Ee) and drug loading (L) in the SLN were calculated from equation 1 and 2-

$Ee = (Wa-Ws)/ Wa \times 100\%$ (1)

L = (Wa-Ws)/(Wa-Ws + Wl)×100 (2)
Where Wa, Ws and Wl were the weight of drug added in system, analyzed weight of drug in supernatant and weight of lipid added in system, respectively.

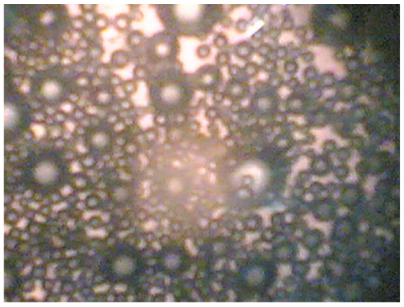
C) FTIR :- To ensure the compatibility of the Nimesulide with lipid preformulation studies were done using IR spectrum recorded on FT-IR by preparing KBr disk. The IR peaks of Nimesulide with the lipids resemble almost same structural peaks of pure Nimesulide indicating the compatibility between the Nimesulide and Glyceryl Monostearate.

D) In vitro drug release[8]: - The time required for dissolution was 12 hr this study was important to confirm that present formulation showed sustained and prolonged release dispersion systems. Much attention was given to investigate release kinetics of Nimesulide from SLNs.

150 mg of drug-loaded SLNs were put into 900 ml of HCl, pH 1.2 at various time points (30 min, 1, 2, 3, 4, 5, 6, 12 hrs), 1ml aliquots were drawn for drug analysis and replaced with an equal volume of dissolution medium. The results were expressed as the percent drug released with respect to the theoretical value.

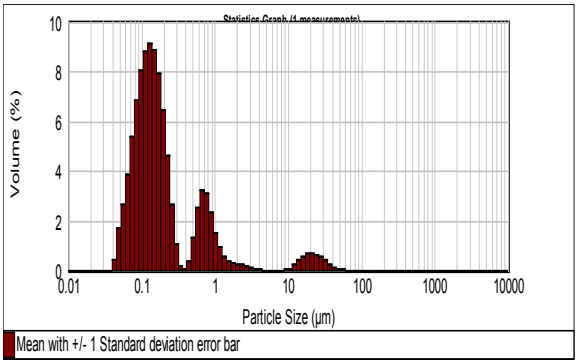
Result and Discussion : -

Particle size :- Particle size of SLN were measured by Malvern zetasizer and were obtained about 300-500 nm which was in confirmation with particle size analysis obtained with homogeneous monolayer coating of surfactant at the periphery of the nanoparticles surrounding the lipid core. Sample was diluted appropriately with the aqueous phase of the formulation for the measurements. The amount of drug bound to the SLN was found to increases with the increase in the GMS concentration and this may be due to the higher intactness of the lipid.

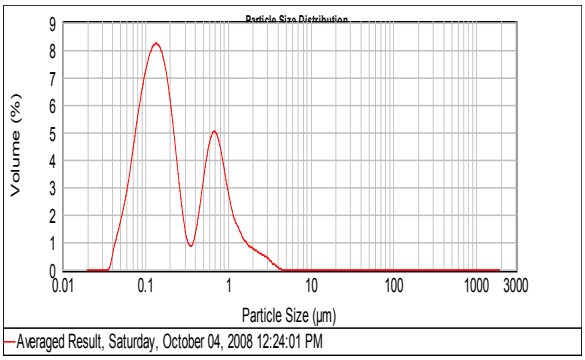


(In a range of 300-500 nm)

Fig. 4 Morphology of Nimesulide loaded SLN (Under Projection Microscope)



a)



(b)

Fig. 5 (a) (b) Particle size Distribution of Nimesulide loaded SLN.

Table 5. Data for EE, DL & PS

Formulations	Entrapment Efficiency (EE) (%)	Drug loading (DL) (%)	Particle Size (PS) (nm)
R1	84.50%	19.00	300-500
R2	67.10%	21.20	550-600
R3	59.37%	20.30	650-750
R4	75.20%	18.90	830-950
R5	76.66%	18.10	600-9700
R6	76.10%	18.30	800-900
R7	63.60%	22.50	900-1000
R8	63.33%	22.40	1000-1100
R9	62.10%	24.20	1030-1150

FT-IR Studies: The IR peaks of Nimesulide with the lipids resemble almost same structural peaks of pure Nimesulide indicating the compatibility between the Nimesulide and Glyceryl Monostearate.

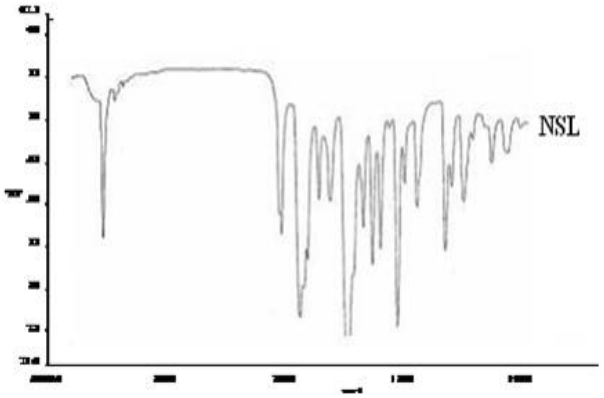


Fig. 6 A) IR Spectra of Nimesulide

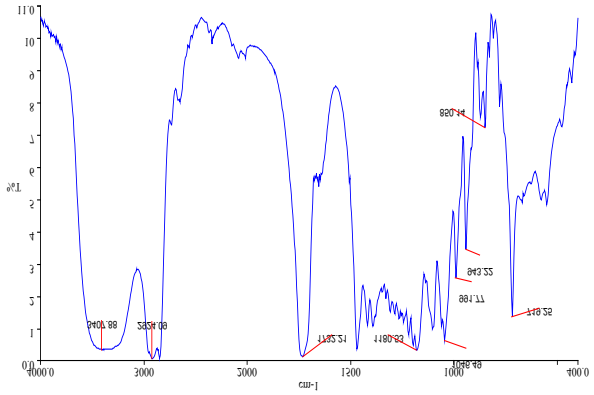


Fig.7: B) IR Spectra of GMS

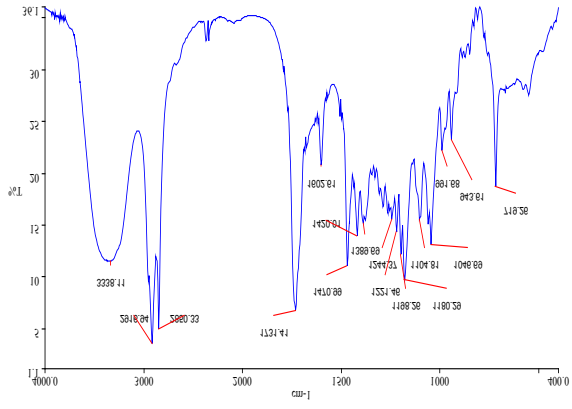


Fig. 8 C) IR Spectra of loaded SLN

***In Vitro* drug release:** The drug released in 0.01 N HCl was 30-50 % in the first 6 hrs and 60-80% during 12 hrs. Drug release was found to be dependant on the concentration of lipid in the formulation. Drug release was found to be slow as concentration of lipid increased.

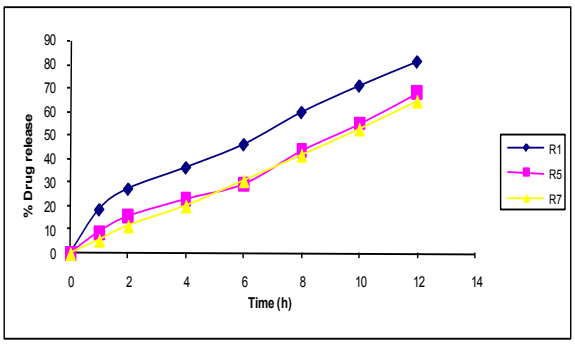


Fig. 9 In Vitro release study of Nimesulide loaded SLN (R1, R5, R7)

In the present study the SLN were prepared according to the emulsion solvent diffusion method using GMS lipid which showed good incorporaton efficiency. The method resulted in consistant production of smaller size nanoparticles in the range of 300-500 nm with narrow size distribution and good entrapment efficiency. The stability data of particles, in vitro release profile indicated controlled release of the drug and excellent physical long term stability was indicated by the slow drug release observed in SGF (simulated gastric fluid i.e 0.01M HCl with pH of 1.2) which could be attributed to the residual PVA associated with SLNs

The formation of homogeneous emulsions with narrow size distribution which results in formation of small particles about 300-500 nm was obtained. And the maximum drug entrapment was found with R1 (84.50%), R5 (76.66%) and lowest entrapment with R3 (59.37%). In vitro release study indicates, dissolution for 12 hrs indicates sustained and prolonged release. Hence a conclusion can be drawn that a systematic formulation approach can be adopted to reach an optimum point in the shortest time with minimum efforts.

Conclusion :-.

Although polymeric nanoparticles and liposomes are efficient NSAID's carriers, the advantage with SLNs is that unlike liposomes, their long-term stability as well as drug incorporation efficiency is better whereas in contrast to polymeric formulations, the risk of residual

organic solvents is minimum. Lipids such as GMS (used for preparing SLNs in the present study) are a part of the regular diet and their intermittent use as a drug carrier is not likely to pose any health hazard. This is in contrast to the use of synthetic polymers whose safety by the oral route must be well documented. Another limiting factor towards the use of synthetic drug carriers is their high cost. However, SLNs were not yet explored for the oral delivery of NSAID's our findings suggest that SLNs offer an economical and patient friendly approach for the administration of NSAID's, bearing a high chemotherapeutic potential.

List of Abbreviations

SLNs- Solid lipid nanoparticles
NSL- Nimesulide
GMS- Glyceryl monostearate
PVA- Polyvinyl alcohol
ESD- Emulsification Solvent Diffusion

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