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Research Article

Formulation Development and Characterization of Solid Lipid Nanoparticles Containing Nimesulide

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Abstract
The aim of this study was to prepare nimesulide solid lipid nanoparticles (NIM-SLNs), to formulate the controlled drug release and to evaluate its physiochemical characteristics. NIM-SLNs were prepared by an emulsification and low-temperature solidification method. Additionally, attempts have been made to study the effect of individual process parameters (stirring speed and stirring time) and formulation parameters (Lecithin concentration, drug concentration and surfactant concentration) on entrapment efficiency. An approximately entrapment efficiency of (60%) and an average drug loading of (1.0 %) were achieved from optimized formulation of NIM-SLNs. The results show that the TMZ-SLNs had an average diameter of 187±1.23nm and *in vitro* drug release was conducted in phosphate-buffered saline (pH 7.4) at 37°C. The cumulative percentages drug release of nimesulide was found approximately 60% in 24 hours and release behavior was in accordance with Higuchi-equation. The results indicate that the SLNs is a promising controlled-release system. It may also allow a reduction in dosage and a decrease in systemic toxicity.

Key words: Solid lipid nanoparticle; Nimesulide.

INTRODUCTION

Nanoparticles made from solid lipids are widely used as colloidal drug carriers for intravenous application.[1-4] The nanoparticles are in the submicron size range (50–1000 nm) and they are composed of physiological lipids. At room temperature the particles are in the solid state. Therefore, the mobility of incorporated drugs is reduced, which is a prerequisite for controlled drug release. They are stabilized with non-toxic surfactants like sodium taurocholate and lecithin.[5] Due to the production by high pressure homogenization they can be produced on large industrial scale. In addition, this production method avoids the use of organic solvents.

Conventional preparation like solution, suspension or emulsion is suffer from following limitation: High dose and low availability, first pass effect, exhibit fluctuations in plasma drug levels and they don't provide sustained effect; therefore there is a need for some novel carriers which could meet ideal requirement of parenteral delivery system. Recently nanoparticles delivery system have been proposed like liposome nanoemulsion, microemulsion, nanosuspension, microparticles, polymeric nanoparticles, nanostructured lipid carriers (NLC) and solid lipid nanoparticles (SLN). But all systems except last two suffer from various limitations. Nanoparticles made from solid lipids is gaining increasing attentions as colloidal drug carriers for intravenous application.[1,4,6,7] In future NIM-SLN can be used as anticancer. SLN not change their organoleptic as well as pharmaceutical feature on aging for almost one year. They are based on biocompatible lipid and provide sustained effect by either diffusion or dissolution.

NIM was introduced in 1985 and it is one of the most potent NSAIDs advocated for use in various inflammatory conditions. It is official in British Pharmacopoeia.[8] 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. Chemically it is [4-nitro-2-(phenoxy) methane sulphoanilide] and has a structure potentially capable of accessing the COX-2 side pocket when the two COX isoforms became organized.[9-10] It is an inhibitor of cyclo-oxygenase 2, hence inhibits the synthesis of destructive prostaglandins and spares cytoprotective prostaglandins. Other than Prostaglandins inhibition it is also inhibit the platelets aggregation. Clinical studies have shown that NIM to be analgesic, anti-inflammatory and antipyretic in a wide range of conditions.[11] Unfortunately, the use nimesulide in clinics is restricted by various contries, which is caused by the accumulation of this drug colitis and oral ulcerations.[12] To avoid treatment-limiting side effects and to get better efficacy, modern therapy requires that the drug reaches the site of action in the most efficient

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Table 1: Experimental design of optimization prescription

Factors	Levels			
	1	**2**	**3**	**4**
Stirring speed (rpm)	500	1000	2000	3000
Stirring time (minutes)	15	30	45	60
Lecithin concentration of (%)	1	5	10	15
Drug concentration (%)	5	10	15	-
Conc. of Sodium. Taurocholate (%)	0.4	0.8	1.6	-

Where Conc. – concentration; sod- Sodium

way, which can be achieved, by using colloidal drug carriers as delivery system.[13-14]

In the present work, we reported the encapsulation of nimesulide using SLNs as the drug carrier. SLNs as colloidal drug carriers combined the advantages of polymeric nanoparticles and liposomes while simultaneously avoiding some of their disadvantages such as acute and chronic toxicity or low stability.[6] So the primary aim of formulation of NIM-SLN is reduce the dose and dosing frequency of nimesulide, because oral and parenteral routes of administration of nimesulide cause various adverse effect. In addition, this study aimed to achieve controlled release of nimesulide, meanwhile avoiding adverse side effects by the conventional formulation. The use of nanoparticles as the drug–carrier system is a very attractive strategy to achieve controlled drug release.[15] A distinct advantage of SLNs over polymeric nanoparticles is the fact that the lipid matrix is made from physiologically tolerated lipid components, which decreases the potential acute and chronic toxicity. Additionally, attempts have been made to study the effect of individual process parameters (stirring speed and stirring time) and formulation parameters (drug concentration and surfactant concentration) on entrapment efficiency as an evaluation index.

2. Materials and methods

2.1. Materials

Pure Drug sample of NIMESULIDE was kind gifted by Signa Pharma, Kanpur (India); Lecithin of medical grade and Sodium taurocholate of medical grade were purchased from Sigma Co. (Germany); Tween 80, from Nice Chemicals, Cochin. All chemicals were either analytical or spectroscopic grade. Distilled water was filtered through a 0.45μm (cellulose acetate) membrane prior to use.

2.2. Optimization of prescription and technology with orthogonal experimental design

In order to study the influence of experimental parameters on the preparation of SLN is based on single factor, five factors which mainly affected the entrapment Efficiency were optimized, and they are following: (1) Effect of Stirring speed; (2) Effect of Stirring time; (3) Effect of concentration of lecithin; (4) Effect of drug concentration; (5) Effect of emulsifier. Individual prescriptions were design, according to single factor variation, in order to screen optimal formulation and further formulation of prescription and artwork of preparation. Based on entrapment efficacy as an evaluation index,[15] the factors and levels of the experimental design are mentioned in Table 1.

2.3. Preparation of NIM-SLNs

The preparation of SLN was based on emulsification and Low-temperature Solidification Method reported by Huang *et al.,*[16] Nimesulide (10 mg) was dissolved in 2ml of Methanol and mixed with 5ml of acetone solution containing 100mg stearic acid and 10 mg lecithin. The mixtures were sonicated and 50 ml of 0.8% (w/v) Sodium taurocholate was added in these mixtures, stirring (Heracus Co. Germany) at 3000 rpm for 30min. The mixed solution was transferred to icy water bath (about 0–2^{o}C) for 4.0 h and continuously stirred in order to form SLNs. The NIM-SLNs suspension was stored at (0-4^{o}C) for long term storage.

2.4. Characterization of SLNs

Prepared SLN dispersion were subjected to light microscope by placing 0.1mL of dispersion on glass slide and observed immediately to see their visible movement. The average particle size and size distribution of the SLN were determined by Photon correlation spectroscopy. The samples of SLNs were diluted to 1:9 v/v with deionized water. The particle size and size distribution are represented by average diameter size. The morphology and average diameter of TMZ-SLNs was examined by transmission electron microscopy (JEM-1200EX, Japan).

2.5. Determination of entrapment efficiency and drug loading

To determine drug entrapment efficiency and loading, the freeze dried SLNs were dissolved in methanol and 7.4 PBS under water bath at 65 ◦C for 30 min and then cooled to room temperature to preferentially precipitate the lipid. Drug content in the supernatant after centrifugation (4000 rpm for 15 min) was measured at 296 nm against the blank by UV-VIS spectrophotometer (Shimadzu 1700). The drug entrapment efficiencies and loading are calculated from following equation (1) and (2).

Percent Drug Entrapment Efficiency =

$$\frac{\text{Actual drug content (mg)}}{\text{Total mass of microspheres}} \times 100$$

Drug loading was calculated as drug analyzed in the nanoparticles versus the total amount of the drug and the excipients added (lecithin, stearic acid and sodium taurocholate) during preparation according to following equation:

Percent Drug Loading =

$$\frac{\text{Analyzed weight of drug in SLNs}}{\text{Analyzed weight of SLNs}} \times 100$$

2.6. Evaluation of in vitro release

In vitro release was evaluated by using a dialysis bag diffusion technique[68] under sink condition. The drug release from NIM-SLNs was performed in PBS (pH 7.4) using dialysis bag. NIM-SLNs suspension was placed in dialysis bags (MW

12000, CO Sigma) and the dialysis bags were subsequently placed in flasks containing 25 ml dissolution medium (PBS pH 7.4) and stirred at 100 rpm in a 37 ◦C water bath. Aliquots of the dissolution medium were withdrawn at each time interval and the same volume of fresh dissolution medium was added to the flask to maintain a constant volume.[17] Drug concentration in the dissolution medium were determined

Table 2: Result of experimental design of optimization prescription

Formulation	Parameter	EE (%)
Effect of Stirring speed (rpm)		
F1	500	38.19±3.71
F2	1000	43.95±3.1
F3	2000	55.11±2.857
F4	3000	59.09±2.801
Effect of stirring time (minutes)		
F5	15	39.92±3.2
F6	30	59.08±2.36
F7	45	57.95±3.03
F8	60	53.32±3.57
Effect of concentration of lecithin (%)		
F9	1	38.19±3.71
F10	5	53.32±3.57
F11	10	59.09±2.801
F12	15	50.37±2.85
Effect of drug concentration (%)		
F13	5	43.01±2.16
F14	10	58.96±3.16
F15	15	50.96±3.35
Effect of concentration of sodium taurocholate (%)		
F16	0.4	51.1±3.16
F17	0.8	59.76±3.58
F18	1.6	50.37±2.85

Values are mentioned in mean±SD, Where SD= standard deviation (n=3); EE (%): percentages of entrapment efficiency

using UV/Vis Spectrophotometry (Shimadzu 1700). All experiments were carried out in triplicates. Results are expressed as means ± standard deviation in table 3 and fig. The release profile of nimesulide from SLNs alongwith the mechanism of the drug release found by inserting the data of NIM-SLNs to curve fitting data of various equation like zero order, first order, Higuchi release and korsmeyer peppas release mechanisms.

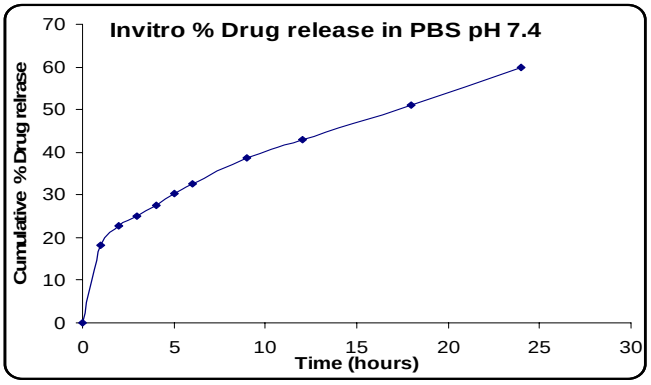

Fig. 1: Evaluation of invitro % release of NIM-SLNs

3. Result and discussion

In the present study various formulations were prepared on the basis of individual factors such as stirring speed (500, 1000, 2000 and 3000 rpm) and stirring time (15, 30, 45 and 60 min.). The formulations were named with code of F1 to F8, Based on the entrapment efficiency of individual formulations. Most efficient formulations were found to be F4 with stirring speed (3000 rpm) and F6 with stirring time (30 min.) Both the formulation were considered to explore further for investigation to see the effect of concentration of lecithin (1%, 5%, 10% and 15%), drug concentration (5%, 10% and 15%) and concentration of sodium taurocholate (0.4%, 0.8% and 1.6%) in SLNs of nimesulide. Further all the formulation were named with code of F9 to F18, again based on entrapment efficiency of individual formulation. Most efficient formulations were found to be F11 with concentration of lecithin (10%) F14 with drug concentration (10%) F17 with concentration of sodium taurocholate (0.8%). All the formulation (F11, F14 and F17) with F4 and F6 were considered for formulation of SLNs. In all the formulation, the entrapment efficiency were found within a range of 35-60%. Accordingly we choose the optimized formulation to prepare the blank and nimesulide loaded Solid lipid Nanoparticles.

The experimental design indicates that concentration of Soy lecithin and sodium taurocholate are two important factors other than drug concentration affecting entrapment efficiency. An average entrapment efficiency of 59.76±3.58 and drug loadind of 1.0% were achieved in nimesulide loaded SLNs with optimized formulation.

Table 3: Evaluation of *Invitro* % release of NIM-SLNs

S. No.	Time (hrs)	√time	Cumulative release (%)	% drug Unreleased
1	0	0	0	100
2	1	1.000	18.19±1.04	81.81
3	2	1.414	22.77±1.05	77.23
4	3	1.732	25.06±0.65	74.94
5	4	2.000	27.57±0.92	72.43
6	5	2.236	30.21±1.28	69.79
7	6	2.449	32.69±0.98	67.31
8	9	3.000	38.78±1.96	61.22
9	12	3.464	43.03±1.06	56.97
10	18	4.242	51.17±0.24	48.83
11	24	4.899	59.90±0.18	40.1

Values are mentioned in mean±SD, Where SD= standard deviation (n=3)

nd on stirring speed and coded with F1 to F4; four formulations based on stirring time and coded with F5 to F8; four formulations based on concentration of lecithin and coded with F13 to F15with F9 to F12; three formulations based on drug concentration and coded with F13 to F15; three formulations based on concentration of sodium taurocholate and coded with F16 to F18. These parameter influence the preparation of SLN and the optimized combinations were selected from various formulations on the Basis of entrapment efficiency as evaluation index; like F4 for stirring speed (3000 rpm), F6 for stirring speed, F11 for concentration of lecithin to stearic acid (10%), F14 for drug concentration to stearic acid (10%) and F17 for concentration of sodium taurocholate (0.8%). The use of 3000 rpm stirring speed resulted in the formation of nano dispersion, characterized by spherical shape

under photon correlation spectroscopy having a mean diameter 187±1.23nm and approximately 60% entrapment efficiency. Stirring time also affect the size of dispersion and entrapment efficiency, smaller and larger stirring time caused lower entrapment efficiency. Thirty minutes were sufficient to produce optimum entrapment efficiency. Concentration of lecithin, drug and Sodium taurocholate was optimized with respected to entrapment efficiency, 10%, 10% and 0.8% of concentration of lecithin, drug and sodium taurocholate was found to be optimum. Nanoparticles systems such as SLN were characterized for various parameters like optical, size, surface morphology, entrapment efficiency and *in vitro* release. All the parameters were showed effective characteristics of SLNs. Entrapment efficiency and drug loading was found approximately 60% and 1.0% respectively.

In vitro dissolution characteristics of nimesulide of other reported article showed higher release. Abdelkader et al.,[18] were showed approximately 40% of nimesulide was released in 2h and Freitas et al.,[19] more than 70% of nimesulide was released within 24 h where as in this work at the 2nd h less than 20% and at 24th less than 60% nimesulide release from the SLNs. The drug release from NIM-SLNs was found in controlled manner, these data were shown the lipophilicity of nimesulide and effectiveness of SLNs, and all the data were summarized in table. The release profile of nimesulide from various SLNs formulations along with the mechanism of the drug release as found by inserting the drug release data of various batches to curve fitting data of various equations like zero order equation, first order equation and Higuchi equation. The drug release from SLNs seems to consist of two phases: an initial rapid release followed by a slower exponential stage. The results obtained until 2h for the in vitro drug release study were not considered because the of burst effect that do not correspond to the real mechanism of the drug release from SLNs. Although the significance of burst release in controlled delivery systems has not been entirely considered, no successful theories have described the phenomenon yet. Despite the fast release of drug in a burst stage is used strategically in certain drug administration, the negative effects caused the burst may be pharmacologically dangerous and non-viable economically. Therefore a through understanding of the burst effect in controlled release systems is undoubtedly necessary. Some authors as Huang and Brazel,[20] were described some experimental observations of burst release in monolithic systems, and theories of the physical mechanisms that cause it. These researchers were the first to think about burst prevention as well as of the treatment of the burst release in controlled release models.

Nanoemulsion or microemulsion suffers from limitations of phase separation; Liposomes are unstable at room temperature and required storage facility. Microparticle have problem of size variation and polymeric nanoparticle lead to toxicity after degradation. Organoleptic and morphology features of SLNs do not change with time and are stable almost one year from production. They are based on physiology tolerated lipids, so risk of toxicity is very less or none. The structure of SLN and their crystalline or amorphous behavior was also characterized by DSC. Distribution of drugs within carrier is crucial point related to solubility of drug and its association with matrix. Because some amount of drug could be either adsorbed on surface of carrier or free in liquid, so the carrier should be tested taking into consideration only the encapsulation drug after separation of the free drug. *In vitro* release tests showed that the NIM-SLNs exhibited sustained release when compared to the conventional nimesulide. In the study of 24h, the nimesulide was release slowly from the SLNs, these data was shown the lipophilicity of nimesulide and effectiveness of SLNs.

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