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Nimesulide Incorporated Lipid Microspheres: an Approach to Targeting and Controlled Release

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Nimesulide, a non-steroidal antiinflammatory drug with very short biological half-life was encapsulated within lipid microspheres by a modified solvent evaporation method with an aim of targeting the drug to the site of inflammation and for controlled release. Physiological lipids such as glyceryl tristearate, cholesterol were used instead of synthetic polymer matrix materials. The prepared lipid microspheres were evaluated with respect to particle size distribution, encapsulation efficiency, *in vitro* release behaviour and *in vivo* antiinflammatory activity in rats. Lipid microspheres with a suitable average particle size of 6-7μm could be prepared with this method. The drug was released continuously over 12 days with no burst effect with a maximum release of 74%. The *in vivo* drug release was investigated in rats, by measuring antiinflammatory activity using cotton pellet granuloma method. A single administration of lipid microspheres showed a better antiinflammatory activity when compared to equivalent multiple administrations of the standard nimesulide solution. The drug concentration was higher in the tissues at the site of inflammation of lipid microspheres treated rats. These results indicated the possibility of drug being released at a controlled rate from lipid microspheres and targeted at the inflamed cells.

Particulate carrier systems, including liposomes, microspheres, nanoparticles, and microemulsions have been used as a means of drug targeting[1]. Lipid microspheres are used in parenteral drug delivery to solubilize water insoluble drugs in a triglyceride core, stabilized by a phospholipid monolayer. Particles of size ranging from 1-7 μm can be injected through intramuscular, subcutaneous or intraperitoneal routes for passive targeting to the reticuloendothelial system[1,2]. Lipid microsphere preparations have been developed for passive targeting to the sites of inflammation and vascular lesions[2]. Nimesulide is a non-steroidal antiinflammatory drug (NSAID) which is particularly useful for patients who have allergic hyper sensitivity to aspirin and other NSAIDs[3].Its plasma half life is just 2-5 h, which calls for frequent administration[4]. It is prescribed in conditions like rheumatoid arthritis, osteoarthritis, degenerative joint conditions, musculoskeletal disorders which may involve long term therapy[5]. This may lead to patient non-compliance due to frequent dosing and an increase in side effects. With a view to overcome these problems, an attempt was made to formulate lipid microspheres of nimesulide. The objective was to prepare a formulation of lipid microspheres from which the drug would be released at a controlled rate and targeted to the site of inflammation.

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MATERIALS AND METHODS

Nimesulide was a gift sample from Emcure Pharmaceuticals Ltd., Pune. All other chemicals and solvents used were of analytical reagent grade and distilled water was used throughout the study. The Institutional Animal Ethics Committee of NGSM Institute of Pharmaceutical Sciences, Mangalore, has approved the animal experimental protocols.

Preparation of lipid microspheres:

Lipid microspheres of nimesulide were prepared by

solvent evaporation method based on the method followed by Reithmeier[6] *et al.* Different formulations were prepared with a drug loading of 2%, 5% and 10% of the weight of lipid. Lipid and lecithin in the ratio 2:1, were dissolved in a suitable volume (10 ml) of dichloromethane containing 0.01% span 20. Nimesulide (10 mg) was incorporated into it as a solid under stirred conditions. The resulting preparation was further emulsified into 17 ml of aqueous phase containing 0.01% Tween 20 and 1% w/v polyvinyl alcohol (PVA), as stabilizer, maintained at 65° for 10 s by using magnetic stirrer. The emulsion formed was poured into a larger volume (245 ml) of an ice cold aqueous phase containing 0.01% Tween 20 and 0.1% w/v PVA and stirred by using mechanical stirrer to allow evaporation of the organic solvent resulting in the precipitation of solid particles. The hardened microspheres were separated from the aqueous phase by filtration, rinsed with water and dried at room temperature. Alternatively drug was also incorporated as a solution. The solvent mixture used to prepare drug solution was polyethylene glycol 400 and water in the ratio 2:1.

Drug content in the microspheres:

The amount of nimesulide entrapped in the different microspheres was estimated by triturating 100 mg of lipid microspheres in a mortar with 2 ml of dichloromethane to break the microspheres. To the above solution, about 5 ml of 0.1 N NaOH was added. The lipid will precipitate but drug remains in solution and it is filtered into 10 ml volumetric flask and the precipitate is once again washed with 0.1 N NaOH and this solution is added to the volumetric flask the volume made up. From this solution 1 ml was taken and diluted to 10 ml with 0.1 N NaOH and the absorbance was measured at 394 nm[7] in a UV/Vis spectrophotometer, using which drug content was calculated.

Determination of the size of the microspheres[8]:

Size analysis was carried out using optical microscopic method with the help of a calibrated eye piece micrometer. The size of around 300 particles was measured and the results were tabulated. Average diameter was calculated.

***In vitro* drug release[6]:**

Lipid microspheres (10 mg, accurately weighed) were suspended in 10 ml of the release medium (3% sodium lauryl sulphate[9] in distilled water) and incubated at 37° in a horizontal shaker water bath. The drug released from the lipid microspheres was determined spectrophotometrically at 394 nm[7].

***In vivo* drug release :**

The release behaviour of the drug formulation *in vivo* was studied by measuring antiinflammatory activity in adult male Wistar rats using the cotton pellet granuloma method as described by Vogel *et al.*[10]. The product that demonstrated maximum drug release was chosen for the antiinflammatory studies. The dose was calculated based on the animal weight for the standard and for the formulation, dose was calculated based on the average rate of drug release which was obtained from the *in vitro* release studies. The rats were divided into 3 groups, each group consisting of 6 animals. One group served as control, second group served as standard (10 mg/kg of nimesulide as solution in a mixture of aqueous polyethylene glycol 400 and water in the ratio 2:1 respectively, as daily intraperitoneal injection), while third group received lipid microspheres containing nimesulide (1.35 g of formulation required to release about 10 mg/kg of nimesulide) once at the beginning of the experiment. Male Wistar rats with an average weight of 200 g were anaesthetized with ether. The back skin was shaved and disinfected with 70% ethanol. An incision was made in the lumbar region. By a blunt forceps subcutaneous tunnel was formed and a sterilized cotton pellet of known weight was placed on both sides in the scapular region. The animals were treated for 8 days. Pellets and the tissues at the site of inflammation were removed after the period of treatment, under ether anaesthesia, and the wound was closed by suturing. The pellets were dried to constant weight. The net dry weight was determined. The weight of granuloma was arrived at by calculating the difference.

The concentration of drug in the tissues at the site of inflammation for all the animals was estimated by spectrophotometric analysis at 394 nm. The drug from the tissues was extracted by triturating the tissues in a clean and dry mortar with 0.1 N NaOH. The absorbance of the extract was measured at 394 nm spectrophotometrically, using extract of the control, as the blank.

RESULTS AND DISCUSSION

Among the 12 preparations, maximum yield (93.2%) and highest drug entrapment (68.1%) was obtained with glyceryl tristearate (SEG_{L1}) microspheres as shown in the Table 1. The shape of nimesulide incorporated lipid microspheres was found to be spherical, as shown in fig. 1. The size of the lipid microspheres was found to be ranging between 6.3-9.3 μm, lowest being for cholesterol microspheres (SEC_{L1}).

TABLE 1: CHARACTERISTICS OF NIMESULIDE INCORPORATED LIPID MICROSPHERES

Lipid used	Sample code	Drug incorporated as	Drug loading (%)	Yield* (%)	Drug* content (%)	Mean size* (μm)	Maximum drug released* (%)
	SEG_{S1}	Solid	2	90.56	66.68	7.92	69.44
	SEG_{S2}	Solid	5	87.53	61.34	8.09	66.69
Glyceryl	SEG_{S3}	Solid	10	86.93	59.41	9.27	64.28
tristearate	SEG_{L1}	Solution	2	93.23	68.09	6.33	74.39
	SEG_{L2}	Solution	5	90.38	65.04	6.71	70.52
	SEG_{L3}	Solution	10	92.73	60.60	7.46	67.13
	SEC_{S1}	Solid	2	88.99	62.93	7.59	68.13
	SEC_{S2}	Solid	5	86.72	60.13	8.07	64.85
	SEC_{S3}	Solid	10	87.76	59.31	8.80	62.99
Cholesterol	SEC_{L1}	Solution	2	91.71	66.04	6.29	73.81
	SEC_{L2}	Solution	5	90.49	64.31	6.64	68.80
	SEC_{L3}	Solution	10	86.49	60.13	7.00	65.99

*Average of three determinations. Percentage yield, drug content, mean particle size and maximum drug release observed for glyceryl tristearate and cholesterol microspheres containing nimesulide in the form of solid or solution with different percentage of drug loading.

From the *in vitro* drug release studies, it was observed that the drug was released continuously over a period of 12 days without any burst effect, releasing a maximum of about 74%, from both glyceryl tristearate and cholesterol microspheres in which the drug was incorporated as solution. The maximum percentage of drug released for all the 12 preparations are shown in Table 1. Further, percentage of drug released was higher in case of 2% drug loaded microspheres than 5% and 10%. Thus among all, because of higher yield and drug content, and maximum drug release, glyceryl tristearate microspheres with 2% drug loading, containing drug as a solution (SEG_{L1}) was considered as the best product, the release profile of which is shown in fig. 2.

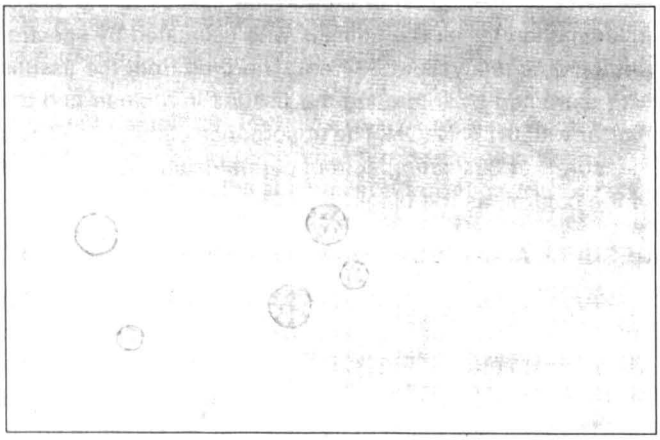

Fig. 1: Photomicrographs of glyceryl tristearate microspheres.

Photomicrographs of glyceryl tristearate microspheres (SEG_{L1}) taken at a magnification of 1000

In order to determine the rate of release, the data fitting was done to zero and first order models for the best product i.e., SEG_{L1} based on the comparative dissolution profiles. The plot obtained with log percentage unreleased versus time was a linear graph indicating that the release occurs according to first order kinetics, but in two phases, initial constant release during six days (rate constant, k= 0.1228 d^{-1}) followed by a slower phase of constant release (k=0.02303 d^{-1}).

The *in vivo* antiinflammatory efficacy was studied for the best product, using the cotton pellet granuloma model,

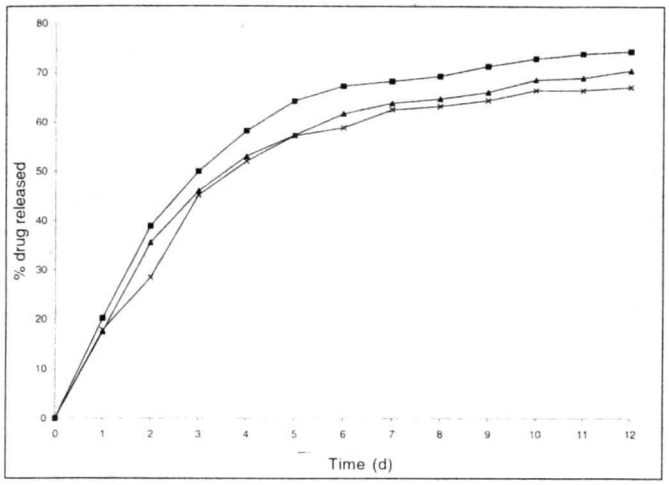

Fig. 2: Dissolution profiles of glyceryl tristearate microspheres.

Dissolution profiles of glyceryl tristearate microspheres. with 2% drug loading (-□-), 5% drug loading (-▲-) and 10% drug loading (-x-), containing drug in the form of solution.

in which inflammation and granuloma develops during a period of several days. This is an indication for the proliferative phase of inflammation. Inflammation involves proliferation of macrophages, neutrophils and fibroblasts, which are basic sources of granuloma formation. Hence a decrease in the weight of granuloma indicates suppression of the proliferative phase[11].

Table 2 shows the effect of drug treatment on the mean weights of granuloma. The lipid microsphere formulation showed 26.2% decrease in the weight of granuloma, whereas, the standard showed a decrease of 20.6%, at a $P<0.001$ compared to control. This indicates that the prepared microspheres exhibited a better efficacy, to the extent of 27% more, than the standard preparation Also the concentration of the drug at the site of inflammation was found to be about 25% more in case of lipid microsphere formulation when compared to standard.

The antiinflammatory effect shown by lipid microspheres for a period of 8 days with a single administration can be considered as a proof of constant release of the drug from lipid micropheres in good correlation with the *in vitro* release pattern. A better efficacy than standard preparation and a higher concentration in the tissues at the site of inflammation of lipid microspheres treated rats support our expectation that the drug is targeted to the site of inflammation. The fact that lipid microsphere formulations are rapidly taken up by macrophages[12] must be the reason for selective accumulation of the drug at the site of inflammation where macrophages are present in large numbers. Thus, incorporating nimesulide into lipid microspheres by solvent evaporation technique, which is simple and reproducible, has shown promising results towards formulation of a target specific and controlled release product.

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TABLE 2: RESULTS OF ANTIINFLAMMATORY ACTIVITY MEASUREMENT

Treatments	Dose (mg/kg)	Weight of Cotton Pellets (mg)		Weight of granuloma (mg)	Percentage decrease in granuloma	Concentration of drug in mg/g tissue at the site of inflammation
		Before	After			
Control	–	20.8±0.09	56.6±0.16	35.8±0.09	–	–
Standard						
Nimesulide	10	20.9±0.18	49.3±0.41	28.4±0.28*	20.6	0.5146±0.0082*
Lipid Microsphere Formulation	Dose equivalent to 10 mg/kg	19.6±0.21	46.6±0.18	26.4±0.12*	26.2	0.6454±0.0083*

Effect of nimesulide incorporated lipid microspheres on cotton pellet induced granuloma. Values are mean±standard error of the mean, Number of data points are 12 (6 animals). *$P<0.001$ when compared to control. Data was analyzed by unpaired Student's t-test.

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