

Formulation and Evaluation of Ibuprofen Loaded Nanoparticles for Improved Anti-Inflammatory Activity

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Abstract:

Methacrylic acid copolymer nanoparticles containing ibuprofen were prepared by emulsion polymerisation technique in continuous aqueous phase. The scanning electron microphotographs showed that the particles were discrete and uniform in size. The drug content and drug recovery of the nanoparticles were studied and it was possible to increase the drug loading capacity by increasing the concentration of the polymer. The interaction between the polymer and the drug were studied by IR spectroscopical studies and it was found that the functional groups responsible for its anti-inflammatory activity was not altered. The *invitro* release studies carried out across the artificial membrane indicated that the release of the drug from the nanoparticles followed zero order kinetics. The study of anti-inflammatory activity on albino rats revealed that nanoparticles of ibuprofen can produce better therapeutic efficacy when compared with the aqueous solution of the drug.

Keywords : Nanoparticles, Ibuprofen nanoparticles, Emulsion polymerization, Sustained release.

Introduction

Nanoparticles are solid colloidal drug delivery systems having the capability to release the drug at an optimum rate at the desired site of action. Nanoparticulate formulations (Chowdary *et al.*, 1997) provide the liberty to use a wide range of polymers like synthetic, natural, biodegradable or non-biodegradable etc. Biodegradable polymers are used for short-term therapy, whereas non-biodegradable polymers (Roland Bodmier *et al.*, 1999) are for long term therapy to administer vaccines and hormones. As the size range of the nanoparticles is 1 to 1000nm, a wide choice for selection of route of administration is also possible. The stability of this formulation is another attraction for the pharmaceutical scientists, while most other novel drug delivery systems like liposomes and niosomes suffer with stability problems, both *invitro* and *invivo*. Nanoparticles were successfully used for delivery of various drugs like cytotoxic, anti-inflammatory (Wehrle *et al.*, 1995) antimalarial agents (Guzman *et al.*, 1993) and immuno suppressive agents. (Cavalloro *et al.*, 1994)

Ibuprofen is a popular non-steroidal anti-inflammatory drug (NSAID) (Pael Insel *et al.*, 1999), which can serve as a model drug for others of its class. Although the drug possesses good anti-inflammatory action, it produces irritation in the gastrointestinal tract when given via peroral route. But if it is given via parenteral route, it is soon eliminated from the vascular system. Hence a pilot experiment was conducted to reduce the dose of ibuprofen as well as to increase the plasma half-life by sustained release techniques using nanoparticulate formulation.

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Materials and Methods

Methacrylic acid copolymer (Eudragit® L100) was obtained from Röhm Pharma, Germany and ibuprofen (99.2 %) was purchased from Sigma Chemical, St. Louis, Mo.

Dichloromethane (E-Merck Ltd., Mumbai), acetonitrile (Ranbaxy, SAS Nagar) were analytical grade and all the solvents used were of HPLC grade.

Scanning Electron Microscope (Hitachi S 450, Japan), FTIR (Perkin Elmer Paragon 2000 series) UV visible spectrophotometer (Elico SL 159, India), sonicator (Vibracell, India), cooling centrifuge (Remi C-24, India), high speed stirrer and magnetic stirrer (Etek, India) were the equipments used at various stages of this studies.

Preparation of nanoparticles: Nanoparticles containing ibuprofen (NPIBU) were prepared by emulsion polymerisation technique in continuous aqueous phase (Katuhiko Ishihara *et al.*, 1991., Fanny De. Jaeghere *et al.*, 1999.) using methacrylic acid copolymer (Eudragit® L100). The methacrylic acid copolymer was dissolved in dichloromethane and this solution was emulsified with a solution of drug containing 2% Tween 80 by stirring for 1 hour at 15°C. The resulting emulsion was then sonicated for 30 minutes at 15°C. The nanoparticles of ibuprofen formed were separated by fractional centrifugation (Meccarron *et al.*, 1998) using a cooling centrifuge and redispersed in phosphate buffer of saline pH 7.4. Three batches of nanoparticles were prepared with varied drug: polymer ratios of 1:1, 1:2 and 1:3 coded as NPIBU-1, NPIBU-2 and NPIBU-3 respectively.

Characterization of ibuprofen nanoparticles: The particle size analysis was carried out by scanning electron microscopy (Hitachi S-450 operated at 20 KV) for five batches and hundred particles were counted at random. Finally an average was calculated from the observed value of five batches.

The drug content was determined by taking 1 ml redispersed NPIBU suspension. To the above suspension, 1ml of acetonitrile was added to precipitate the polymer. To this, 1 ml of aqueous potassium dihydrogen phosphate solution (30 Mm) was added and the mixture was centrifuged at 16,000 rpm (Remi C-24 cooling centrifuge) and 15°C. The clear supernatant was removed and analysed spectrophotometrically (Elico SL 159) at 221 nm and the drug content and drug recovery (Niwa *et al.*, 1994) were calculated.

Infrared spectroscopical analyses were carried out to study the mechanism of attachment of the drug to the polymer and also the intactness of the attachment. The IR spectrum of pure drug, pure polymer and NPIBU formulation were taken and were interpreted and compared with each other.

Invitro release studies: The prepared NPIBUs equivalent to 2 mg were transferred to dialysis tubes with an artificial membrane. To this, 10 ml of phosphate buffer pH 7.4 was added and subjected to dialysis by immersing the tube to a receptor compartment containing phosphate buffer pH 7.4 (250 ml). The receptor compartment was agitated constantly using a magnetic stirrer and the temperature was maintained at 37° C. For 24 hours at different time intervals, 5 ml samples were withdrawn from the receptor compartment and the concentration of the drug released was determined spectrophotometrically at 221 nm (Loganathan *et al.*, 2000). After each withdrawal, equal volume of fresh buffer pH 7.4 was added to the receptor compartment. The studies were also carried out for pure drug for 6 hours

In vivo studies : The anti-inflammatory activity of the NPIBU-3 was carried out in white albino rats by carrageenan induced oedema (Winter *et al.*, 1962). The rats weighing 100-200g were used throughout the studies and were divided into three groups including a control group-receiving placebo. One of the other two groups was treated with NPIBU-3 (drug equivalent to 25mg/kg orally) while the other with the same dose of free ibuprofen aqueous solution. Carrageenan (1%) in saline was injected in a volume of 0.1 ml into the subplantar tissues at the hind paw of the rat. Groups of rats were pretreated with drug 5 hours prior to carrageenan

injection. The foot volume was measured in unanaesthetized rats by plethysmographic method as described by Singh and Ghosh (Singh *et al.*, 1968).

Results and Discussion

Three batches of nanoparticles were formulated with constant amount of drug but with varying ratios of polymer. The scanning electron microscopy of the nanoparticles is shown in Fig.1. The particles were morphologically spherical in shape and discrete. The size range of particles were 150 ± 30 nm, 300 ± 50 nm and 700 ± 50 nm for NPIBU-1, NPIBU-2 and NPIBU-3 respectively. No drug crystals have been identified. The increase in polymer ratio resulted with an increase in the particle size. The increase in polymer concentration might have caused an increase in the concentration of it in the emulsification medium. This condition facilitates the diffusion of more monomers into the micelles, which might have caused a considerable increase in particle size.

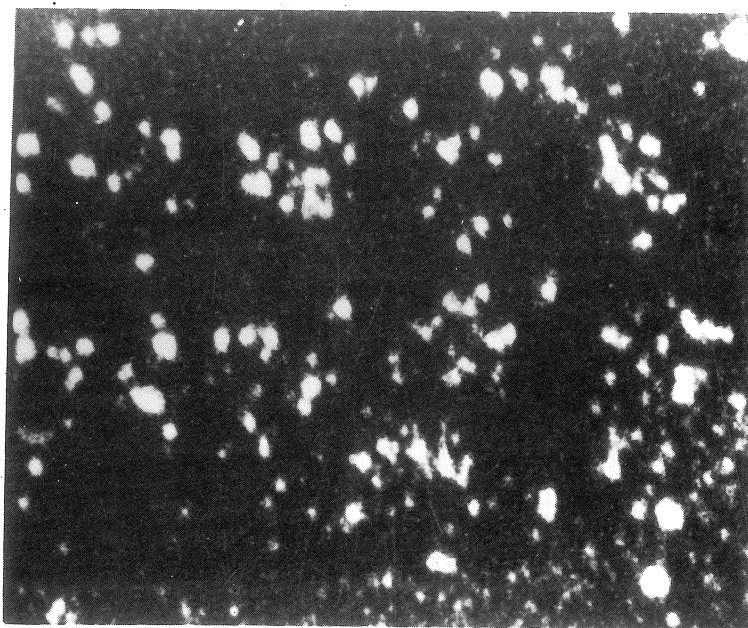


Figure 1. Morphological structure of ibuprofen loaded poly (methacrylic acid) nanoparticles.

The drug content was determined by centrifugation method and was maximum in NPIBU-3 as 56.3 %, while it decreased to 38.4 % in NPIBU-2 and 21.6 % in NPIBU-1 (Table 1). The drug: polymer ratio was not increased beyond 1:3, because the particle size increased proportionately with increase in polymer ratio and the formulation cannot be considered as nanoparticles. These results do not correlate with the results of the study carried out by (Niwa H. *et al.*, 1994) to increase the drug content by increasing the molecular weight of the PLGA copolymer.

The IR spectroscopical studies indicated that the functional group responsible for the anti-inflammatory activity of the drug remained unaltered, hence the drug was expected to get dispersed on the polymeric matrix of the nanoparticles. The increase in the drug content with an increase in the polymer ratio also suggests physical adsorption of the drug on to the polymer.

Table 1. Characterization of nanoparticles of ibuprofen.

Batch Code	Drug – polymer ratio	Particle size (nm)	Drug content (%)	Drug Recovery (%)
NPIBU – 1	1:1	150 (± 30)	21.6	38.9
NPIBU – 2	1:2	300 (± 50)	38.4	51.2
NPIBU – 3	1:3	700 (± 50)	56.3	60.4

The *invitro* release studies carried out across an artificial dialysis membrane are shown in Fig.2 and it clearly indicated that the NPIBU possesses a good sustained release action. The release of the drug from all the three formulations followed a biphasic pattern. An initial burst release of 38.1 %, 43.4 % and 45.6 % was exhibited at the 5th hour by the formulations NPIBU-1, 2 and 3 respectively. The initial burst release may be due to the release of the drug from adsorption sites on the surface of the nanoparticles and the subsequent slow release may be due to the leaching of the drug from the inner matrix of the polymeric particles. All the formulations released 90% or more at the end of the 20th hour whereas the pure drug was released up to 98.6% within 6th hour. The maximum release was achieved from NPIBU-3 and it is about 96.7% at the end of the 24th hour. It is to be noted that the drug content of NPIBU-3 is 56.3% which is approximately double to that of NPIBU-1.

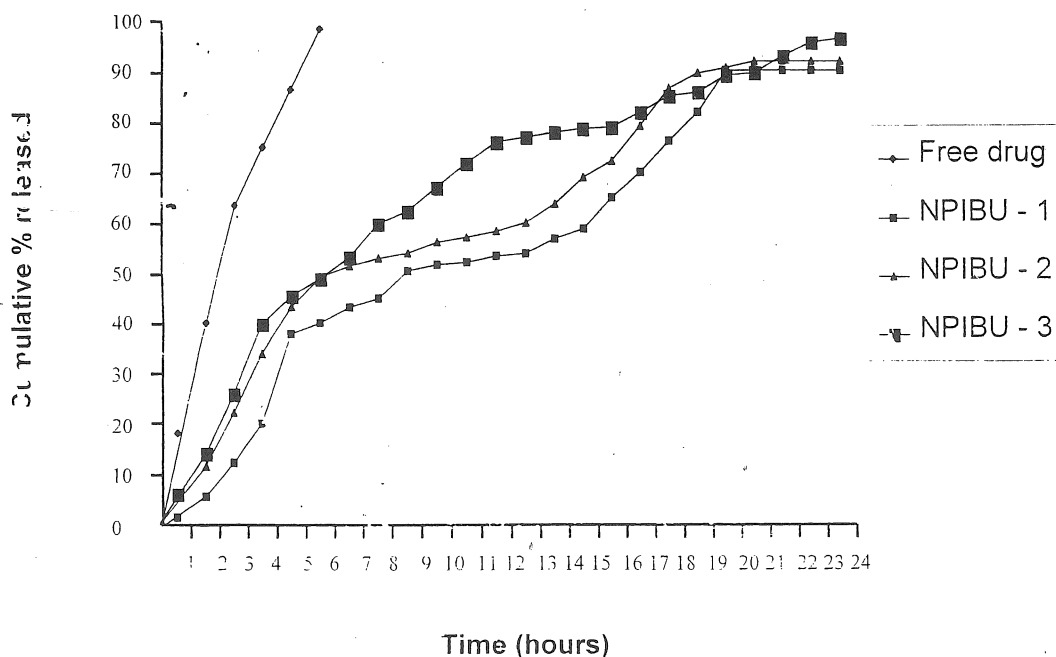


Figure 2. Release of ibuprofen from methacrylic acid copolymer nanoparticles across artificial dialysis membrane.

The *invivo* anti-inflammatory activity tested in albino rats indicated that the NPIBU-3 possesses a sustained release of the drug even after 10th hour, whereas the free drug was less effective after the 6th hour. The graph, percentage oedema vs time shown in Fig.3 confirms the quicker elimination of the free drug when compared to that of the formulation. The group of rats that were treated with placebo was considered as standard oedema and assumed as 100 % oedema, for the comparison of other two groups which were treated with aqueous drug solution and NPIBU-3 respectively. The results of the *invitro* release studies correlates well with *invivo* studies and the formulation exhibits a zero order kinetics. The comparison of the release of ibuprofen from the nanoparticles to that of the free aqueous drug solution was worth observing. The free drug could reduce the oedema only for 5 hours from the time of administration, but the paw oedema was reduced even after 10 hours from the administration of NPIBU-3 formulation. This clearly indicates that the drug was slowly released from the polymeric matrix of nanoparticles in the *invivo* conditions also.

Based on the above studies it can be concluded that nanoparticles can serve as a successful sustained drug delivery system for ibuprofen.

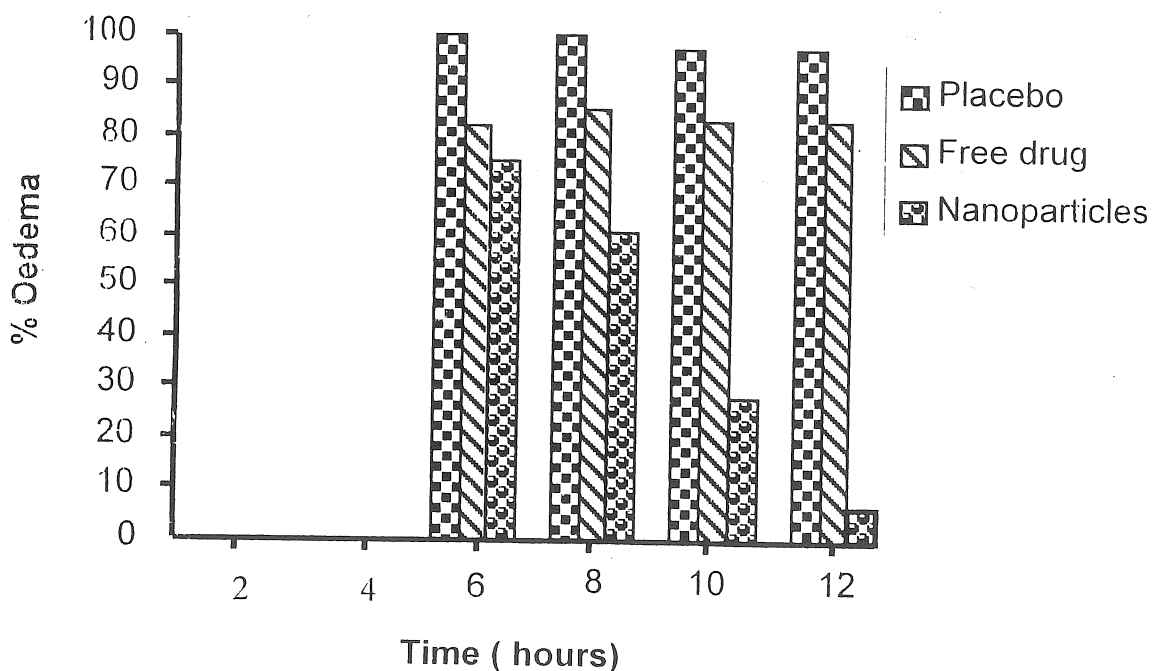


Figure 3. Comparison of the anti-inflammatory activity of NPIBU –3 with aqueous drug solution

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