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Original Research Article

Formulation and *In vitro* Evaluation of Ibuprofen-Loaded Poly(D,L-lactide-co-glycolide) Microparticles

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Abstract

Purpose: *To enhance and control the release of ibuprofen from poly(D,L-lactide-co-glycolide) (PLGA) microparticles.*
Methods: *Ibuprofen-loaded microparticles containing PLGA were formulated using a emulsification/solvent evaporation method. Various concentrations of ibuprofen (200, 300, 400 and 0 mg) were loaded into the PLGA microparticles and the formulations labeled A, B, C and D, respectively. The microcapsules were characterized for drug loading, particle size, polydispersity index, zeta potential (ZP) and drug release.*
Results: *The zeta potential of the microparticles were -53, -68.7, -43.1, and -37.4 mV for batches A, B, C and D, respectively. Polydispersity index ranged from 0.745 to 0.900. Encapsulation efficiency (EE %) and loading capacity (LC) ranged from 83.4 to 89.3 and 23.4 to 30.1, respectively. Maximum and minimum release of 92 and 72.0 % at 18 h were obtained for batches C and A, respectively.*
Conclusion: *The study shows that PLGA-loaded with ibuprofen can serve as an alternative carrier for controlled release of ibuprofen.*

Keywords: *Ibuprofen, Microparticles, Controlled release, Zeta potential, Polydispersity*

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INTRODUCTION

Ibuprofen is an NSAID that is commonly consumed for the treatment of pain and inflammation, the use of which is limited by its low bioavailability [1]. Ibuprofen have been shown to exhibit serious dose limiting toxicities and hypersensitivity reactions, which are a direct consequence of the formulation technique and the absence of selectivity for target tissues [1,2]. Furthermore, the pharmacological effect of this drug has important limitations *in vivo*, related to its very short physiological half life, poor water solubility and the need for subjects to take doses of up to 1 to 2 g/day to obtain the desired therapeutic effects [3].

One major therapeutic strategy is enhancing the solubility and bioavailability of the drug. Several techniques to improve the solubility, bioavailability, control the release and prolong the shelf-life of drugs have been evaluated. These include; particle size reduction by milling or micronization to increase the specific surface area [4], modification of the structure of particles by increasing the amorphous fraction or modification of the polymorph type to promote a faster dissolution [5]. The use of excipients such as surfactants [6], formation of physical

complexes [8,9], dispersion of the drug in carriers [9] and polymeric drug delivery using polyester groups [10]. Polymeric drug delivery systems have proved to be more promising than other carrier systems based on available literature [10-11]. Polymeric microparticles (MPs) offer suitable means for delivering drugs by enhancing bioavailability of the drug via enhanced solubility and high encapsulation efficiency. In addition, polymeric microparticles (MPs) have shown some advantages such as improved stability, good biocompatibility, non-toxicity, biodegradability, and controlled delivery [11,12]. Poly(D,L-lactide-co-glycolide) (PLGA) is a biodegradable synthetic polymer frequently used in drug delivery of insulin and other poorly water soluble drugs [11]. Results indicate that effective protection and controlled release of the drugs can be achieved [11,12]. The aim of the present work was to formulate ibuprofen microparticles using PLGA as a lipophilic polymer and characteristerize them for their physicocmemical properties including in vitro release.

EXPERIMENTAL

Materials

The following material were used in this study: Ibuprofen (Spectrum, USA), ethanol (Fisher, UK), Poly (lactic-co-glycolic) acid (PLGA, copolymer ratio, 50:50) and polyvinyl alcohol (PVA) with molecular weight of 22,000 (Acros Organics, USA). Dichloromethane (DCM) was obtained from Sigma Aldrich, St. Louis, MO. All other reagents used in the study were of analytical grade.

Preparation of the ibuprofen microparticles

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buprofen microparticles (MPs) were prepared by emulsification/solvent evaporation method. Approximately 300 mg of PLGA (50:50) was dissolved in 5 ml of methylene chloride and 200 mg of Ibuprofen was dispersed in the polymer solution. This dispersion was emulsified into the aqueous continuous phase (100 ml) containing 400 mg of polyvinyl alcohol and 100 mg of sodium oleate. The coarse emulsion formed was further homogenized with an Ultra-Turrax® (T18 IKA, Germany) homogenizer at 7000 rpm for 5 min. The resulting microparticles were collected by centrifugation, washed with water and dried at room temperature. The above procedures were repeated using 300 and 400 mg of Ibuprofen and were labeled as (A, B and C). Microparticles containing no drug (unloaded microparticles), which served as the control, were also prepared to contain the same PLGA and PVA and labeled as (D).

Characterizations of the loaded microparticles

Determination of yield of microparticles

The MPs formed were filtered from the solvent, dried in the desiccator and weighed to obtain the yield of the MPs formulated per batch using Eq 1:

Yield (%) = {W1/(W2+W3)} …….. (1)

where W1 is the weight of MPs formulated (g), W2 the weight of drug added (g) and W3 is the weight of carrier (g) used as the starting material.

Fourier transform infrared analysis (FT-IR)

The drug or polymer or drug-loaded PLGA formulations were mixed with solid potassium bromide (KBr). The mixture was then pressed into a very thin disc. The disc were placed in the holder directly in the IR laser beam. Spectra were recorded using Shimadzu FTIR-8400s loaded with IR solution software (version 1.2). IR spectra of pure drug and polymer (PLGA 50:50) were compared with IR spectra of drug –loaded PLGA for any major interaction.

Morphological study

A 5.0 mg quantity each of the samples was weighed and placed on a microscopic slide and mixed with two drops of distilled water, using a glass rod. The mixture was covered with a cover slip and viewed under X 10 objective and X 4 eye piece of binocular light microscope (model 746862, Wetzler, Germany) to which a digital photographic camera had been fixed and connected to a computer. Three distinct regions of the slide were photographed and printed directly from the computer.

Determination of encapsulation efficiency (EE)

A 50 mg quantity of microparticles was dispersed in 50 ml of phosphate buffer (pH 7.4). The dispersion was allowed to stand for 2 h after which it was mixed in a vortex mixer for 10 min and then centrifuged at 5000 rpm for 10 min. The amount of ibuprofen contained in the various microparticulate formulation samples was determined using HPLC. Ibuprofen encapsulation efficiency (EE) was then determined using Eq 2

EE% = ADC/TDC × 100 …… (2)

Where EE% is Ibuprofen encapsulation efficiency, ADC is the actual drug content and TDC is the theoretical drug content.

Loading capacity (LC)

LC expresses the ratio between the entrapped drug by the PLGA (carrier) and the total quantity of the carriers used in the formulation, and is computed as in Eq 3.

LC% = TQDE/TQDC × 100…….. (3)

where TQDE is the total quantity of drug entrapped in the carrier and TQDC is the total quantity of the carrier.

Evaluation of micrometric properties of microparticles

The micrometric properties of the microparticles formulation were investigated by measuring the angle of repose of drug-loaded microparticles using fixed-base cone method. The bulk and tapped densities were measured in a 20-mL graduated measuring cylinder. The compressibility indices of the particles were also evaluated using the equation below. Briefly, the microparticles were filled into the measuring cylinder and tapped mechanically by means of constant velocity rotating cam with the change in its initial bulk density to a final tapped density when there was no further decrease in the packed volume. (ie, unchanged). For statistical purpose, each experiment was carried out in triplicate.

BD = MP/BV ……..…… (4)

where BD is bulk density, MP is mass of the powder and BV is bulk volume.

TD = MP/TV …….. (5)

where TD is tapped density, MP is mass of the powder and TV is tapper volume.

Tan θ = h/r ……..…….. (6)

where is Angle of repose

CI % = [(TD-BD)/TD] × 100 ……... (7)

where CI is compressibility index in %, TD is tapped density, BD is bulk density.

Particle size determination

Particle sizes were determined by Photon correlation spectroscopy (PCS) at 25 ^{o}C using a Multi Angle Particle Size Analyser (Zetasizer 3 Model AZ6004, Malvern England) modified with a 35 mW He–Ne laser (Model 127-35, Spectra Physics USA). The detection was performed at a scattering angle of 90o in a cell AZ equilibrated at 293K and at an accumulation time of 240 s.

Measurement of zeta potential

The zeta potentials of the formulated microparticles were determined after 1 week of preparation in a Zetasizer Nano Series (Nano-ZS, Malvern Instruments England). Each sample was diluted with bidistilled water and the electrophoretic mobility determined at 25 ^{o}C and dispersant dielectric constant of 78.5 and pH of 7. The electrophoretic mobility values were used to calculate the zeta potentials using DTS Version 4.1 software (Malvern, England) and applying Henry's equation as used an earlier studied [13,14]

UE = [2εZf(Ka)]/3η ……. (8)

where Z is the zeta potential, UE is the electrophoretic mobility, ε the dielectric constant, η the viscosity of the medium and f(Ka) is the Henry's function.

***In vitro* drug release studies**

The *in vitro* release profiles of the ibuprofen-loaded microparticles were determined by filling hard gel capsules with a 100 mg quantity of the ibuprofen-loaded microparticles was filled into hard gelatin capsules. Each capsule was then placed in a 250 ml beaker containing 150 ml of phosphate buffer solution (pH 7.4). Agitation of the fluid system at100 rpm was done with a magnetic stirrer. At predetermined time intervals, 0.5 ml samples were withdrawn and replaced with phosphate buffer solution to maintain sink condition for a period of 8 h. The temperature of the dissolution system and the replacement fluid were maintained at 37 ± 0.5 ^{o}C. The ibuprofen content of the withdrawn sample was determined using the HPLC quantitative method. The experiments were performed in triplicate.

Stability studies

The stability of the microparticles was evaluated after 10 months of storage using batch C that shows maximum release within 18 h of the study period. Approximately 1.0 g of the batch was packed in an airtight clean plastic bottle at room temperature (RT), 37 °C, 25°/65% relative humidity, and 45 °C/75 % relative humidity (RH), in environmental test chamber (Modern Industrial Corporation, INDIA). The microparticles were

evaluated for their drug content and physical change such as colour changes in the weight.

Statistical analysis

The data were expressed as mean ± SEM; statistical analysis using SPSS, version 14.0 (SPSS Inc, Chicago, IL, USA) was performed by one-way ANOVA followed by Student t-test. $P < 0.05$ was considered as significant.

RESULTS

The result of the FT-IR (spectra not shown) revealed that the positions of the characteristic absorption bonds for different functional groups and bond of the pure drug, PLGA and the microparticles formulation were not changed considerably, an indication that there was no interaction between the pure drug and the polymer used in the preparation of the microparticles.

The morphology of the microscopic images of the MPs (not shown) indicate that when the ratio of drug to PLGA 50:50 used in formulating the MPs was low, the MPs produced were irregular in shape but when this ratio was increased, more slightly spherical and rough particles were produced.

Results in Table 1 show the flow indices of ibuprofen-loaded PLGA 50:50 formulations. The values obtained indicate poor flow properties with exception of batch A, indicates that, addition of sodium oleate did not have any effect on the flow properties. Usually, powders with good flow properties should have angle of repose < 25 ^{o}C, Hausner's ratio < 1.25, and Carr's index < 20 %. The results here revealed angle of repose, Hausner's ratio, and compressibility indices for all the formulations range between 20 – 26 ^{o}C, 1.37–1.43, and 27.3 – 30.3 %, respectively.

The particle size distribution and zeta potential of the microparticles presented in Table 2 shows low-sized uniform microparticles were obtained, except for microparticles prepared without drug (batch D). The data in Table 2 shows that increase in concentration of the drug reduced the particle sizes. Particle sizes of 99.6, 91.2 and 78.8 µm were obtained for batch A, B and C respectively, while that of the control (batch D) shows 124.1 µm in size. However, the sizes of the formulation were close, but the polydispersity indices (PI) were significantly different ($p < 0.05$), the microparticles show PI of 0.745, 0.785, 0.800 and 0.91 for A, B, C and D, respectively. The mean values of the zeta potential (mV) of A, B, C and D formulations (-53,-68.7, -43.1 and-37 mV) respectively, were all negative. The high negative values of zeta potentials obtained in these formulations are due to the presence of uncapped end carboxyl groups.

Drug loading and encapsulation efficiency

The results of the EE (%) (Table 2) show that drug EE (%) increased with increase in the amount of ibuprofen up till 400 mg for all batches, yielding maximum EE % of 89.00, 84.60 and 83.00 % for microparticles formulated with PLGA (A-C), respectively. So, the microparticles loaded with 400 mg of ibuprofen resulted in higher EE %, while those loaded with 200 mg ibuprofen gave the least. All the batches had good EE% (83–89 %). Table 2 also shows that maximum LC of 29.5, 30.10 and 23.40 % of ibuprofen were obtained for A, B and C, respectively. Thus, formulation containing 300 mg of ibuprofen gave higher LC than those containing 200 mg or 400 mg of ibuprofen.

In vitro release of ibuprofen

The *in vitro* dissolution profiles are shown in Figure 2. From the results, at T1 (1 h) 46.34, 32.51, 29.32 and 51.10 % of ibuprofen were released from batches A-C and the standard ibuprofen tablet (batch D). At T6 (6 h), about 66.37, 60.3, 61.00 and 73.00 % of ibuprofen were released from batches A-C, and the reference ibuprofen tablet (batch D).

Table 1: Some micromeritics properties of ibuprofen microparticles formulations

Batch	FR (g/s)	BD (g/cm^3)	TD (g/cm^3)	AR (^{o})	HI	CI (%)
A	1.29 ± 0.14	0.42 ± 0.25	0.58 ± 0.12	23.94 ± 0.07	1.38 ± 0.08	27.3 ± 0.06
B	1.31 ± 0.15	0.43 ± 0.08	0.62 ± 0.04	20.11 ± 0.09	1.43 ± 0.01	30.3 ± 0.04
C	1.30 ± 0.03	0.44 ± 0.08	0.60 ± 0.06	26.56 ± 0.03	1.43 ± 0.07	29.9 ± 0.03
D	1.30 ± 0.08	0.33 ± 0.23	0.44 ± 0.02	28.16 ± 0.22	1.38 ± 0.43	28.2 ± 0.12

Key: *FR= flow rate, BD= bulk density, TD= tapped density, AR= angle of repose, CI = compressibility index; HI = Hausner's index; batches A, B and C contain 200, 300, 400 mg of ibuprofen respectively, while batch D does not contain ibuprofen*

Table 2: Some characterization of microparticles prepared with PLGA loaded and unloaded with Ibuprofen

Batch	PDI	ZP (mV)	LC (%)	EE (%)	PS(µm)	PY (%)
A	0.745	- 53.01	29.51 ± 0.4	83.41 ± 0.2	96.60 ± 0.4	85.23
B	0.785	- 68.17	30.12 ± 0.1	84.31 ± 0.1	91.12 ± 1.4	87.13
C	0.800	- 43.12	23.40 ± 0.2	89.56 ± 0.4	78.81 ± 0.2	91.49
D	0.910	- 37.24	-	-	72.13 ± 0.1	86.88

PDI = polydispersibility index; ZP = zeta potential; LC =loading capacity, EE = encapsulation efficiency; batches A, B and C contain 200, 300, 400 mg of ibuprofen, respectively, while batch D does not

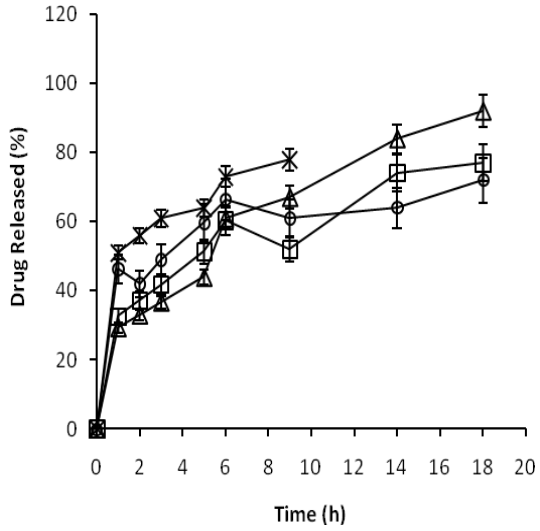

Figure 1: Release profile of Ibuprofen loaded microparticles: 0 = A, □ = B, Δ = C, and ×= MK, contain 200, 300, 400 mg ibuprofen and commercial sample, respectively

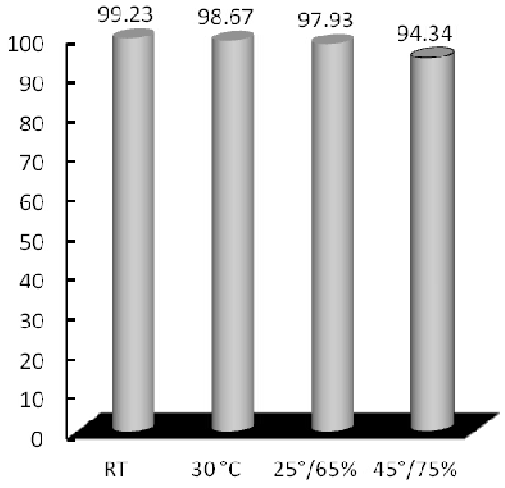

Figure 2: Time-resolved stability data at various storage conditions (for batch C): RT= room temperature; RH = relative humidity

Also, at T9 (9 h), it was observed that 61.12, 52.24, 67.65 and 78.10 % of ibuprofen were released from batches A-C, and the market ibuprofen tablet (batch D) and finally at T18 (18 h), 72.11, 77.21, and 92.34 % of ibuprofen were released from batches A - C. The formulations exhibited good sustained release properties required for once daily administration. And, maximum release was found in batch C at the end of the release time study. It shows that the release of the drug was drug concentration-dependent.

Figure 2 depict the results of the drug content as a function of the stability of the preparation. It indicates that there was no significant ($p > 0.05$) from the initial basal concentration, which was taken as 100 %.

DISCUSSION

Solvent evaporation techniques have been exploited widely to encapsulate drugs in polymeric delivery systems. The flow property of the formulation was poor with the exception of batch A, which shows relative good flow, an indication that addition of glidant is needed to enhance the flow property of the preparation. The particle sizes were quite close and considerably good enough for microparticles. Particle size may be a function of either one or more of the following: formulation excipients, degree of homogenization, homogenization pressure, rate of aggregation of particle, crystalline habit of the particle etc. From the particle size result, it was observed that batch A and C had the largest and smallest particle size among the loaded MPs, respectively. The unloaded microparticles (MPs) show smaller particle size than the loaded MPs.

Polydispersity index (PI) measures the width of the particle size distribution. Consistently high values of PI indicate either an aggregated or poorly prepared sample as shown in some of the batches of the microparticles. If the PI is above 0.5, the PI loses its significance as an accurate measure of the width of the size of distribution, but can still be useful for comparative purposes. Low PI (less than 1) of any preparation is an indication of a better control of particle size [15]. The high negative values of the zeta potential obtained were due to uncapped end carboxyl

groups. The loaded formulations (A, B and C) showed a different result by possessing higher values than the unloaded formulation (batch D). This indicate that the presence of ibuprofen in the loaded batches was not able to shield the surface charge, thereby causing a shift in the shear plane inward instead of outwards from the particle surface.

The results of the *in vitro* release of ibuprofen from the MPs showed that the preparations exhibited higher sustained release properties significantly difference ($p < 0.05$) from the reference or market sample. The formulations gave a gradual and more sustained release of the drug over the study period. The results showed that ibuprofen-loaded MPs could be used once daily for the treatment of arthritis and some other inflammations as the case maybe at the recommended dose. However, initial burst release was observed for ibuprofen-loaded microparticles in the first hour, which was probably due to the rapid dissolution of the drug adhered onto the surface of the microparticles. Though, burst release may not be completely discouraged especially in conditions such as arthritis, as the initial amount of drug released can serve as a loading dose, while the subsequent release may serve as maintenance dose [8,15].

Figure 2 showed the drug content from the microparticles formulation after exposure to different storage conditions. The results indicate that, there was no significant change in the drug content as well as the physical appearance of the drug-loaded microparticles after 10 months of storage.

CONCLUSION

Based on the results obtained, the ibuprofen-loaded MPs exhibit good physicochemical properties for oral dosage administration as well as satisfactory sustained release properties for once daily administration. Therefore, ibuprofen-loaded MPs based on PLGA would be for the development of suitable oral controlled release of ibuprofen.

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