Ibuprofen Nanoparticles for Oral Delivery: Proof of Concept

Catarina Pinto Reis1, João Pinto Ferreira2, Sara Candeias1, Cátia Fernandes1, Nuno Martinho2, Natália Aniceto3, António Silvério Cabrita4 and Isabel V. Figueiredo2

1CBIOS – Laboratory of Nanoscience and Biomedical Nanotechnology (LNBN), Lusophone University of Humanities and Technologies, Lisboa, Portugal
2Laboratory of Pharmacology and Therapeutic Care, CEF, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal
3Lusophone University of Humanities and Technologies, Lisboa, Portugal
4Experimental Pathology Laboratory, Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Abstract

Background: Using nanoparticles to improve non-steroidal anti-inflammatory drugs therapeutic profile is an interesting approach, especially concerning their gastric toxicity.

Objective: The aim of this work was to present a proof of concept for a nanoparticulate formulation composed of a biodegradable polymer poly (DL-lactic acid) (PLA) meant for oral delivery of ibuprofen (IBU) to systemic circulation with reduced gastric toxicity.

Materials and methods: IBU-loaded nanoparticles composed of PLA and poloxamer 188 were prepared by an emulsion/solvent diffusion method. The particles obtained were characterized for size, zeta potential and morphology, as well as encapsulation efficiency. Nanoparticles were given to Wistar rats at an equivalent dose of 12 mg/kg (i.d.) of ibuprofen for a period of 10 days. Both concentration of IBU in the plasma and toxicity in different tissues were evaluated.

Results: Nanoparticles displayed a size of 281.1 ± 66.7 nm with a zeta potential of -4.3 mV. Scanning electron microscopic images showed spherical shape particles with low polydispersity index. IBU concentration in blood samples indicated that nanoparticles were able to deliver IBU to systemic circulation. A significant reduction in toxicity was observed for nanoparticles in gastric mucosa compared to free ibuprofen. This may be due to controlled release of IBU from the nanoparticles, which decreases the mucosal contact to IBU. In summary, we designed a proof of concept for PLA nanoparticles as suitable carrier for IBU allowing reduced gastric toxicity of the drugs. This strategy can eventually be applied to other non-steroidal anti-inflammatory drugs.

Keywords: Poly (Lactic Acid) (PLA); Nanotechnology; Gastric toxicity; Wistar rats; Non-steroidal anti inflammatory drugs (NSAIDs)

Introduction

Ibuprofen, 2-(4-Isobutylphenyl)propionic acid, is a NSAID that inhibits the cyclooxygenase system. It is generally used as analgesic and anti-inflammatory for a variety of inflammatory pathologies [1]. IBU can be used as short duration therapy (e.g. headache) or as chronic therapy, such as in osteoarthritis and rheumatoid arthritis. Although IBU has poor water solubility (~ 1 mg/mL) [2], when given orally it is well absorbed (approximately 100%), with a peak plasma concentration around 1-2 hours after ingestion [3,4]. However, it is rapidly eliminated from systemic circulation displaying a relatively short half-life (1.7-2 h), and therefore, requiring several dosages for an effective and prolonged pharmacological activity [5]. Furthermore, when taken for chronic diseases, the maintenance of analgesic effect is more important than the rapid onset of action. Considering all this, different exposures to IBU are expected and consequently, are associated to different risks for adverse effects. Despite being better tolerated in comparison to other NSAIDs [1], it has demonstrated NSAIDs-related gastric toxicity, including gastric irritation and bleeding, abdominal pain and ulcers [6]. Hence, to achieve effective and prolonged drug levels for an extended period without having related gastric effects, IBU is a potential candidate for a new formulation based on controlled and sustained release.

Various strategies have been explored to avoid NSAIDs-related toxicity, including concomitant administration of gastric protectors (as free-drugs or coupled) [7], use of rectal drug delivery systems [8], or modified release formulations [9]. The development of oral controlled and sustained release offers a potential benefit for NSAIDs.

The rationale behind it is allowing the release of the drug at a desired rate, providing sustained levels (fewer doses required) and reducing the contact with gastric mucosa (reduced gastric damage). To this end, polymeric nanoparticles have raised increased attention due to their properties and benefits for drug pharmaceutical performance (site-specific targeting and controlled release) [10].

Nanoparticles are solid colloidal delivery systems capable of releasing optimum amounts of drug, while avoiding premature release. Also, due to their small size, they are able to be absorbed through the oral mucosa to reach systemic circulation [11]. The possibility of using biocompatible and biodegradable polymers is another advantage, since the vehicle itself is then removed naturally without promoting toxicity [12].

Poly(lactic acid) (PLA) is a biocompatible and biodegradable linear aliphatic polyester that is regarded as safe [12]. PLA has been extensively

*Corresponding author: Catarina Pinto Reis, CBIOS-Laboratory of Nanoscience and Biomedical Nanotechnology (LNBN), Lusophone University of Humanities and Technologies, Portugal, Tel: +351217515550; Fax: +351-21-7515598/79; E-mail: catarina.reis@julusofona.pt

Received September 05, 2013; Accepted October 30, 2013; Published November 04, 2013

Citation: Reis CP, Candeias S, Fernandes C, Martinho N, Aniceto N. et al. (2013) Ibuprofen Nanoparticles for Oral Delivery: Proof of Concept. J Nanomedicine Biotherapeutic Discov 4: 119. doi:10.4172/2155-983X.1000119

Copyright: © 2013 Reis CP, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
studied for drug delivery applications [13-15]. Due to its insolubility in water, it provides a good platform to easily produce nanoparticles, and is suitable for the encapsulation of poor water soluble molecules, such as IBU. As a result, it is expected that the IBU-loaded PLA nanoparticles will exhibit high loading capacity with a controlled release of IBU and a decrease in gastric irritation.

The aim of the work herein described was to develop IBU-loaded PLA nanoparticles as a proof of concept of the value of this formulation as a delivery systems for NSAIDs, in general. In order to do so, we assessed, through in vivo studies, its ability to reduce gastric toxicity and other NSAIDs-related toxicity and verified if IBU is able to reach systemic circulation.

Materials and Methods

Materials

Ibuprofen and Poloxamer (POLX) were obtained from Sigma-Aldrich (Steinheim, Germany). PLA (Purasorb PDL02) was obtained from PURAC (Barcelona, Spain). Benzophenone was obtained from Schlarau (Barcelona, Spain). All chemicals or solvents were of analytical grade.

Nanoparticle preparation

Ibuprofen nanoparticles were prepared by a method of spontaneous emulsification/solvent diffusion (SESID), as generally described in the literature [16]. Briefly, 4 g of PLA and 50 mg of IBU were dissolved in ethyl acetate (2%, v/v) and saturated with water (for a total of 200 mL). The emulsification was produced by the addition of the previous solution to 400 mL of a saturated aqueous solution of ethyl acetate with POLX at 5% (w/v) under stirring for 5 minutes at 11,000 rpm. Then, an excess of water was added to the emulsion under moderate stirring (400 rpm) for 5 minutes. The solvent was evaporated under reduced pressure at 40°C. The remaining solution was then centrifuged and the obtained pellet was used for further experiments.

Characterization of IBU-loaded PLA nanoparticles

Determination of particle size and zeta (ζ) potential: The particle size and zeta potential were measured in Delsa™ Nano C (Coulter Beckman, USA) by photon correlation spectroscopy (PCS) and electrophoretic mobility, respectively. Both measurements of size and zeta potential were performed after centrifugation and re-suspension of the nanoparticles in distilled water. Each analysis was carried out at 300×g for 10 minutes and then analyzed. HPLC separation was performed under isocratic conditions at room temperature. The method acid (80:20, v/v). The eluent was monitored at a wavelength of 264 nm and methanol (to a final volume of 2 mL). The mixture was centrifuged at 300×g for 10 minutes and then analyzed. HPLC separation was performed with a 20 µL injection volume on a LiChroCART 250-4 RP18 column using a mobile phase composed of methanol/phosphoric acid (80:20, v/v).

For evaluation of the type of lesions in the esophagus and gastric mucosa, a criterion was established, as shown in Table 1. Each tissue sample was scored based on found lesions, according to an arbitrary grading system. The ulcer index (UI) for each stomach and esophagus was the sum of scores of all lesions and reported as median (minimum-maximum). The significance of differences between groups was assessed and p<0.05 versus control was taken as significant.

Determination of IBU in rat’s plasma by HPLC: IBU concentrations in plasma were analyzed by HPLC [18], solely to demonstrate the formulation’s ability in allowing quantifiable systemic drug concentrations. From the blood samples taken, 0.5 mL of plasma was treated with 20 µL of benzophenone (internal standard reference) and methanol (to a final volume of 2 mL). The mixture was centrifuged at 300×g for 10 minutes and then analyzed. HPLC separation was performed with a 20 µL injection volume on a LiChroCART 250-4 RP18 column using a mobile phase composed of methanol/phosphoric acid (80:20, v/v).

Animals received either 12 mg/kg (3 times a day) of IBU (suspended in aqueous medium), or an equivalent dose of IBU encapsulated into PLA nanoparticles. Following 10 days of treatment after the last administration, rats were sacrificed and blood samples were collected. The blood was transferred to eppendorf tubes with 10 µL of heparin sodium salt to prevent blood clotting. Samples were then centrifuged at 4000 rpm for 5 minutes and the plasma was transferred into another eppendorf tube and kept in the cold until HPLC analysis. On the other hand, stomachs, esophaguses, livers and kidneys were isolated and rinsed with saline, measured, weighted and prepared for histological analysis. Therefore, the organs were cut into transverse fragments (5 mm) and immersed in a 10% formaldehyde solution for 24 h. The pieces were processed into paraffin and stored at -20°C. The fragments were cut in 4 µm pieces in a SHANDON (AS 325) microtome and stained with Hematoxylin and Eosin. The resulting images were obtained and observed using a NIKON (Eclipse E600) microscope.

For in vivo studies, rats were dosed with two months old) were divided into 3 groups: (i) one group dosed with ibuprofen-loaded-nanoparticles (n=7); (ii) the second group dosed with free ibuprofen aqueous solution (n=3); (iii) the third group dosed with phosphate buffer pH 7.4 (control) (n=2). A single rat receiving water was kept as normal control. Rats were weighted on a daily basis and allowed free access to water and food on a 12 h light/dark cycle. All formulations were given orally and studies were conducted in conformity with the animal ethics guidelines for care and use of laboratory animals and approved by ethics committee.

In vivo study design

In vivo studies were performed to evaluate the ability of nanoparticles to deliver IBU to systemic circulation and their ability to prevent IBU-related gastric toxicity. Wistar rats (male with 200-300 g with two months old) were divided into 3 groups: (i) one group dosed with ibuprofen-loaded-nanoparticles (n=7); (ii) the second group dosed with free ibuprofen aqueous solution (n=3); (iii) the third group dosed with phosphate buffer pH 7.4 (control) (n=2). A single rat receiving water was kept as normal control. Rats were weighted on a daily basis and allowed free access to water and food on a 12 h light/dark cycle. All formulations were given orally and studies were conducted in conformity with the animal ethics guidelines for care and use of laboratory animals and approved by ethics committee.

Table 1: Criterion to evaluate toxicity in different organs.

| Type of lesion                  | Score of lesion severity |
|--------------------------------|--------------------------|
| Normal tissue                  | 0                        |
| Inflammation                   | 0.5                      |
| Loss of mucosal folds          | 1                        |
| Hemorrhagic areas              | 1.5                      |
| Ulcers/necrotic areas          | 2                        |
| Perforation                    | 3                        |

Citation: Reis CP, Candeias S, Fernandes C, Martinho N, Aniceto N, et al. (2013) Ibuprofen Nanoparticles for Oral Delivery: Proof of Concept. J Nanomedine Biotherapeutic Discov 4: 119. doi:10.4172/2155-983X.1000119
Results

Characterization of the formulation

The IBU-loaded PLA nanoparticles showed homogeneous distribution (polydispersity index of 0.1), without any noticeable aggregation from the PCS analysis and SEM, with a mean particle size of 281.1 ± 66.7 nm and a zeta potential of -4.3 mV. Such surface charge is expectable due to the high POLX content comparatively to the PLA content. The zeta potential is a function of the surface charge, and therefore, is a predictor value of the stability of the particles. Although particles had a low zeta potential which is normally associated with instability (aggregation due to Van Der Waals inter-particle attraction). SEM analysis (Figure 1) showed well-defined smooth surface and spherical particles, without noticeable aggregation, caused possibly by the presence of POLX as a steric stabilizer [19]. Furthermore, since zeta values close to zero may be undesirable, we could argue the possibility of lowering the amount of POLX used, in order to obtain higher negative zeta potential. However, even PLA particles with stronger surface charge (ranging from -40 to -55 mV) have also been associated to gastric instability due to flocculation [19], thus this alternative scenario would also be undesirable. Moreover, no free drug crystals have been identified and this particulate system achieved high encapsulation efficiency, being able to retain 84 ± 5% of the initial amount of IBU.

In agreement to our results, various methods have been reported to produce PLA nanoparticles with small size and low polydispersivity [16,20,21]. Among them, the SESD method, as used here, is a choice methodology since it is simple to perform, has high batch-to-batch reproducibility and low-energetic input to form nanoparticles [22]. The encapsulation by SESD is based on the dissolution of the polymer with subsequent precipitation of the same, by diffusion of the solvent to the aqueous phase [16]. The POLX acted as stabilizer of the nanoparticulate system, in order to modulate the size and surface properties, as well as stability of nanoparticles.

In vivo study in rats-toxicity assessment

The amenability of PLA nanoparticles as a carrier for IBU was further investigated in vivo after oral administration. The IBU levels were evaluated in plasma and NSAIDs-related toxicity was evaluated in the different organs macro- and microscopically. As observed by HPLC analysis (Figure 2), IBU was detected in blood samples taken from rats receiving IBU-loaded PLA nanoparticles (1 from Figure 2) and free IBU (2 from Figure 2), while controls did not show any peak of IBU (3 from Figure 2). However, the concentration peak in blood from rats receiving IBU-loaded PLA nanoparticles was smaller than that reached with free IBU. This difference could be attributed to the polymeric network that could have retarded the release of IBU, therefore suggesting a controlled...
Moreover, due to their small size, nanoparticles are expected to be uptake to systemic circulation. In fact, the size of the particles obtained is in the range for good oral absorption, especially by M cells in Peyer’s Patches [11]. Since IBU is poorly soluble in water at physiological pH, nanoparticles could have resulted in a reservoir from which IBU would slowly diffuse.

The various organs were examined and no differences were observed in weight and size. However, slight differences in esophagus and stomach morphology could be observed between groups receiving free IBU and IBU-loaded PLA nanoparticles (Table 2), revealing a slightly higher U.I. in the group receiving IBU. NSAIDs-related toxicity occurs mainly at the levels of the gastric epithelium. As it can be observed (Table 2 and Figure 3A), major macroscopic alterations were observed in the stomach. For rats receiving IBU-loaded PLA nanoparticles (Figure 3A), the macroscopic morphology was maintained to some extent. However, for rats receiving free IBU, the structural morphology was completely altered (complete loss of gastric mucosal surface), with evidence of bleeding hemorrhagic focus, as well as ulcers (Figure 3B). In some cases, all mucosal surfaces showed to be edematous with inflammatory infiltrates. Histological analysis of stomach (Figure 4A and 4B) corroborates the decrease of IBU-related toxicity in the stomach mucosa when IBU was administered in nanoparticle dosage form. High amounts of infiltrated red cells were observed for free IBU, while with IBU-loaded PLA nanoparticles, these infiltrations were very low. Moreover, the latter showed no evident hemorrhage and the maintenance of gastric pits was observed, which is a sign of substantially reduced IBU toxicity. The same pattern was observed for the esophagus, although to a lesser extent. Complete loss of the epithelium was observed for all rats receiving IBU, while in rats receiving IBU-loaded PLA nanoparticles, this effect was slightly decreased.

Figure 5 shows the histological images of liver and kidney for control animals and animals treated with free IBU and IBU-loaded PLA nanoparticles. No differences were observed between the control and test group. The images from the liver show well shaped hepatic cells, with no signs of blood stasis in the extracellular medium or necrotic cells (Figure 5B). Also, the kidney's histological analysis reveals no differences between control and test group (Figures 5D-5E). Together, these results reveal the absence of liver and kidney toxicity of IBU-loaded PLA nanoparticles.

Results provide evidence of cause-effect relationship between IBU uptake and gastric injuries, observed by the absence of damage in the control group, and that PLA nanoparticles could offer an advantage of protection against NSAIDs-related gastric toxicity. The lack of statistical significance between I.U. of free and encapsulated drug can probably be explained by the fact that one single rat in the IBU-NP

| Experimental group | Product(s) administered (p.o.) | Dose | Ulcer index (U.I.) [median (min-max)] |
|--------------------|-------------------------------|------|-------------------------------------|
| IBU-NP group n=7   | IBU loaded-nanoparticles      | 12 mg/kg | 1.6 (4.5-0.0) 0.9 (2-0) |
| IBU group n=3      | IBU in aqueous solution       | 12 mg/kg | 2.0 (2.5-1) 1.0 |
| Control group n=2  | PBS 7.4                       |      | 0 0.0 0.0 |
| Normal rat n=1     | water                         |      | 0 0.0 0.0 |

Table 2: Ulcer index in the stomach and the esophagus in the different groups studied.

Figure 3: Stomach morphology after intake of (A) IBU-loaded PLA nanoparticles; (B) free IBU and (C) Control.

Figure 4: Microscopic analysis of stomach after intake of (A) IBU-loaded PLA nanoparticles; and (B) free IBU. Scale bars indicate 5 mm.

Figure 5: Histological study of liver and kidney. Staining with hematoxylin (cell nuclei-blue) and eosin (cytoplasm-pink/red). A-C: Microscopic analysis of liver: Negative control group (A); Animals treated with IBU-loaded PLA nanoparticles (B); Animals treated with free IBU (C); 20x magnification in the original, Bar=50 µm. D-F: Microscopic analysis of kidney: Negative control group (D); Animals treated with IBU-loaded PLA nanoparticles (E); Animals treated with free IBU (F); 40X magnification in the original, Bar=25 µm.
group showed much higher tissue damage than the rest of the group. However, it should be noted that the IBU-NP group presented animals without any lesion both in the stomach and the esophagus, contrarily to the IBU group, which is indicative of the gastro-protective potential of this formulation.

We hypothesize that PLA allowed the reduction of gastric toxicity by avoiding the direct contact between gastric mucosa and the drug by retardation of its release. Since IBU belongs to the class II drugs of the BCS, this means that the limiting step for its uptake will be the release from the polymeric matrix. The reduced uptake of IBU in the stomach by nanoparticles could, therefore, have a major impact in patients taking chronic therapeutics. The fact that PLA is hydrolysable in aqueous environment and the degradation products are biocompatible, represents an extra advantage, which was confirmed by the fact that no toxicity non-related to IBU was found. Other polymers have been reported to encapsulate NSAIDs to alter their delivery. As an example, IBU-loaded PLGA nanoparticles [24] and Eudragit L100 nanoparticles [4] allowed the controlled release of IBU. Others [2] reported diethylaminomethyl-dextran IBU nanoparticles allowed a pH sensitive, burst release. Furthermore, nanocapsules with indomethacine effectively prevented intestinal lesions [9].

As intended from the established goals, this study is in fact a proof of concept for a formulation that will allow IBU administration with reduced gastric toxicity. This would ultimately increase IBU efficacy and safety, offering a major advantage over conventional formulations in improving clinical outcome. Furthermore, these results offer the possibility of using PLA nanoparticles for an extended number of drugs of the same group.

Declaration of Interest

The authors also would like to thank to Prof. Dr. Lia Ascensão from FCUL (University of Lisbon) for her kind technological support in electron microscopy experiments.

References

1. Sweetman S (2009) Martindale: The Complete Drug Reference. Pharmaceutical Press, London, UK.
2. Jiang B, Hu L, Gao C, Shen J (2005) Ibuprofen-loaded nanoparticles prepared by a co-precipitation method and their release properties. Intl J Pharm 304: 220–230.
3. Zhu KJ, Li Y, Jiang HL, Yasuda H, Ichimaru A, et al. (2005) Preparation, characterization and in vitro release properties of ibuprofen-loaded microspheres based on poly(lactide, poly(ε-caprolactone) and their copolymers. J Microencapsulation 22: 25-36.
4. Kumar S, Rajikumar S, Ruckmani K (2003) Formulation and evaluation of ibuprofen loaded nanoparticles for improved anti-inflammatory activity. Acta Pharm Turcica 45: 125-130.
5. Potthast H, Dressman JB, Junginger HE, Midha KK, Oesper H, et al. (2005) Biowaiver monographs for immediate release solid oral dosage forms: Ibuprofen. J Pharm Sci 94: 2121-2131.
6. Moore N (2007) Ibuprofen: A journey from prescription to over-the-counter use. J Roy Soc Med 100: 2-6.
7. Borhade N, Pathan AR, Halder S, Karwa M, Dhiman M, et al. (2012) NO-NSAIDs: nitric oxide-releasing prodrugs of non-steroidal anti-inflammatory drugs. Chem Pharm Bull 60: 465-481.
8. Ozguney I (2011) Conventional and novel pharmaceutical dosage forms on prevention of gastric ulcers. Peptic Ulcer Disease. InTech Pp 323-350.
9. Raffin RP, Obach ES, Mezzalira G, Pohlmann AR, Gutierrez SS (2003) Nanocapsulas poliméricas secas contendo indometacina: Estudo de formulacao e de tolerância gastrointestinal em ratos. Acta Farm Bonaerense 22: 163-172.
10. Reis CP, Neufeld RJ, Ribeiro AJ, Veiga F (2006) Nanoencapsulation II. Biomedical applications and current status of peptide and protein nanoparticulate delivery systems. Nanomed Nanotech Biol Med 2: 53-65.
11. Des Rieux A, Fievez V, Gairnot M, Schneider YJ, Preat V (2006) Nanoparticles as non-steroidal oral delivery systems of proteins and vaccines: A mechanistic approach. J Control Rel 116: 1-27.
12. Kumari A, Yadav SK, Yadav SC (2010) Biodegradable polymeric nanoparticles based drug delivery systems. Colloids Surf B 75: 1-18.
13. Hans ML, Lowman AM (2002) Biodegradable nanoparticles for drug delivery and targeting. Curr Opin Solid State Mater Sci 6: 319-327.
14. Tobio M, Sanchez A, Via A, Soriano I I, Evora C, et al. (2000) The role of (PEG) on the stability in digestive fluids and in vivo fate of PEG-PLA nanoparticles following oral administration. Colloids Surf B 18: 315-323.
15. Musumeci T, Ventura CA, Giannone I, Ruosi B, Montenegro L, et al. (2006) PLA/PLGA nanoparticles for sustained release of docetaxel. Intl J Pharm 325: 172-179.
16. Reis CP, Neufeld RJ, Ribeiro AJ, Veiga F (2006) Nanoencapsulation I. Methods for preparation of drug-loaded polymeric nanoparticles. Nanomed Nanotech Biol Med 2: 8-21.
17. Essa S, Rabanel J M, Hidgen P (2010) Effect of polyethylene glycol (PEG) chain organization on the physicochemical properties of poly(D,L-lactide) (PLA) based nanoparticles. Eur J Pharm Biopharm 75: 96-106.
18. Rustum Y (1997) Assay of ibuprofen in human plasma by rapid and sensitive reversed-phase high-performance liquid chromatography: application to a single dose pharmacokinetic study. J Chromatogr Sci 29: 19–20.
19. Reich G (1997) In Vitro stability of Poly(D,L-lactide) and Poly(D,L-lactide)/poloxamer nanoparticles in gastrointestinal fluids. Drug Dev Ind Pharm 23: 1191-1200.
20. Bilati U, Allemann E, Doelker E (2005) Development of a nanoprecipitation method intended for the entrapment of hydrophobic drugs into nanoparticles. Eur J Pharm Sci 24: 67-75.
21. Mosqueira V, Legrand P, Pinto-Alphandary H, Puisieux F, Barratt G (2000) Poly(D,L-lactide) nanoprecipitates prepared by a solvent displacement process: Influence of the composition on physicochemical and structural properties. J Pharm Sci 89: 614-626.
22. Murakami H, Kobayashi M, Takeuchi H, Kawashima Y (1999) Preparation of poly(dl-lactide-co-glycolide) nanoparticles by modified spontaneous emulsification solvent diffusion method intended for the entrapment of hydrophilic drugs into nanoparticles. Eur J Pharm Sci 24: 67-75.
23. Schliecker G, Schmidt C, Fuchs S, Kissel T (2003) Characterization of a homologous series of d,l-lactic acid oligomers: A mechanistic study on the degradation kinetics in vitro. Biomaterials 24: 3835-3844.
24. Abidin SZ, Sameni J, Julianto T, Bukhari NI (2009) In vivo evaluation of ibuprofen-loaded Poly(lactico-co-glycolico) acid nanoparticles in rats. AIP Conf Proc 1139: 171-175.