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Etodolac Loaded Poly (Lactide-Co-Glycolide) Nanoparticles: Formulation and *In Vitro* Characterization

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Introduction

Nanoparticles are drug carriers for targeted delivery, with a size range about 10 and 1000 nm. They diffuse in the body very well but can be recognized by the human body as foreigner intruders, so easily opsonized and removed from the blood circulation[1,2]. Nanoparticulate delivery systems have some advantages including to control drug release profiles, prolonging the presence of drugs in blood circulation, and to target drugs to a specific site [3,4].

Poly (d,l-lactide-*co*-glycolide), (PLGA) and PLGA based polymers are widely used in pharmaceutical formulations. Indeed, these polymers are approved by the Food and Drug Administration because of their biocompatibility and biodegradability.[5] Many studies are about PLGA-carriers as delivery systems for a variety of therapeutic substances like anticancer agents, antibiotics,[6] nonsteroidal anti-inflammatory drugs [6], vaccines,[7] macromolecules [4,8]. PLGA is a water-insoluble polymer with strength hydrophobicity [5], most of them under amorphous form than crystalline and they dissolve in a wide range of solvents like acetone, ethyl acetate, chlorinated solvents.

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PLGA is obtained by the polymerization of glycolic and lactic acids, linked by ester linkages as seen in Figure 1. PLGA polymers can be formulated into different ratio of monomeric units which can influence the time of degradation. This degradation is due to the hydrolysis of the ester linkages and release nontoxic monomers. So the risk of long-term toxicity or the risk of an immunological reaction is minimized [9].

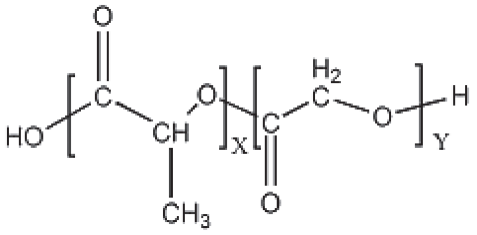

Figure 1
Chemical structure of PLGA (x: number of lactic acid units, y: number of glycolic acid units)

Etodolac is a non steroidal anti-inflammatory drug which is effective in treating fever, pain, and inflammation in the body. It is an inhibitor of cycloxygenase which belongs to the pyranocarboxylic acid group (Figure 2) [10,11]. Edotolac is a racemic mixture of [+]S and [-]R enantiomere. It is a white crystalline compound, practically insoluble in water but soluble in alcohol, chloroform, dimethyl sulfoxide and aqueous poly ethylene glycol [12]. As many NSAIDs, etodolac has side effects, as gastrotoxicity, cardiovascular risk. Formulation of etodolac loaded nanoparticles may reduce these side effects and help to target the active substance for a better efficacy.

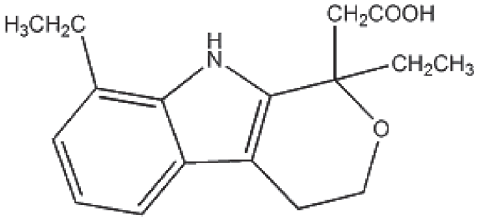

Figure 2
Chemical structure of etodolac

The objective of this study was to develop and characterize nanoparticulate drug delivery systems for etodolac using PLGA. Nanoparticles have been produced by the nanoprecipitation method.[13,14] Nanoparticle formulation was evaluated in terms of particle size, zeta potential, entrapment efficiency and drug release profiles.

Materials and Methods

Materials

PLGA (50:50, Mw: 40 000-70 000) was purchased from Sigma Aldrich (St. Louis, USA). Etodolac was provided from Eczacıbaşı (Turkey). Pluronic F68 was obtained from ICI Surfactants (Clamart, France). Acetone was purchased from Riedel-de Haen (Seelze, Germany). PEG was obtained from Merck-Schuchardt (Hohenbrunn, Germany). Ultra pure water (Milipore Simplicity 185, France) was used to prepare nanoparticles.

Preparation of PLGA Nanoparticles

The nanoparticles were prepared by nanoprecipitation method similar to that employed by Fessi et al. (Figure 3).[13,14] Briefly, 75 mg of PLGA were dissolved in 5 mL of acetone. This organic phase was poured into 15 mL deionized water containing 75 mg of Pluronic F-68 and 1 mg etodolac with moderate stirring at room temperature. Nanoparticles were immediately formed and acetone was then removed from the colloidal suspension by rotoevaporation under reduced pressure and nanosphere dispersion was obtained.

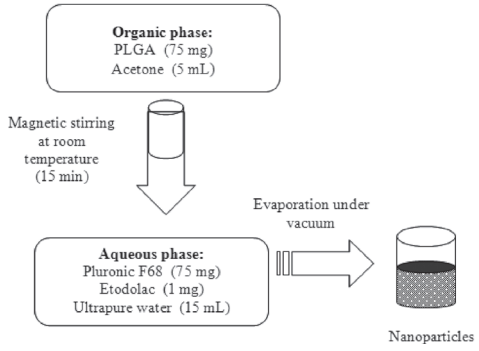

Figure 3
Nanoprecipitation method used for the preparation of nanoparticles

Characterization of PLGA Nanoparticles

Particle Size Analysis

Mean particle size of the nanoparticles was determined by Photon Correlation Spectroscopy (PCS) with a Malvern Zetasizer Nano-ZS (Malvern Instruments, Malvern, UK). Measurements were realized in triplicate at a 90º angle at 25ºC under suitable dilution conditions. Particle size distribution was expressed as mean diameter (nm) ± standard deviation and polydispersity index.

Zeta Potential Measurement

Zeta potential of nanoparticle dispersions was measured in mV by Malvern Zetasizer Nano-ZS (Malvern Instruments, Malvern, UK) in triplicate to determine the surface charge and the potential physical stability of the nanosystem. Zeta potential of nanoparticles was measured in aqueous dispersion. Measurements were realized in triplicate at a 120º angle at 25ºC.

Scanning Electron Microscope (SEM) Analysis

A SEM (Jeol-SEM ASID-10. Device in 80 KV, Japan) was used to evaluate surface characteristics of the nanoparticles. Nanoparticles were mounted on the metal stubs with conductive silver paint and then coated with a 150A° thick layer of gold in a Bio-Rad sputter apparatus. SEM images of the samples were obtained at different magnifications.

Drug Loading and Yield

Loaded drug quantity was determined according to the following procedure: unbound drug was separated by centrifugation (Hermle Z-323 K, Germany) at 5000 rpm for 15 min. Supernatant was then collected and lyophilized. Afterwards, the precipitate was dissolved in methylene chloride, 1 mL PBS (pH 7.4):PEG_{400} was added and extracted for 2 h. After the evaporation of methylene chloride, the polymer was removed, the solution was filtered (Swinnex-GS, Millipore, UK) and etodolac content inside the nanoparticles was detected by UV spectrophotometer at 280 nm.

The total nanoparticle yield was calculated gravimetrically on the basis of polymer/drug recovery.

In Vitro Drug Release

Nanoparticle samples (50 mg) were suspended in 1 mL pH 7.4 PBS:PEG_{400} in a ratio of 60:40 providing sink conditions in polypropylene vials and agitated 50 rev.min^{-1} at 37ºC in a thermostated bath system (Memmert, Schwabach, Germany) at 37°C. At each time intervals, after centrifugation at 13.500 rpm during 10 minutes at room temperature, the supernatant was removed and replaced with equal volume of fresh release medium maintained at the same temperature. Drug content was evaluated using a UV spectrophotometer at 280 nm.

Results and Discussion

Nanoparticles were prepared with nanoprecipitation technique. This technique was first developed and patented by Fessi and co-workers [13]. It is a rapid and easy technique and nanoparticles were formed spontaneously. It requires two miscible solvents and ideally both the polymer and the drug must dissolve in the first one, but not in the second solvent. The solvents nature, the solvents volume and the polymer concentration are important for the size and shape of nanoparticles. Nanoprecipitation enables the production of small nanoparticles (100–300 nm) with narrow unimodal distribution.

Nanoparticles were characterized by particle size distribution, zeta potential analysis, drug entrapment efficiency and in vitro release studies.

Particle Size Analysis

Nanoparticles were characterized in vitro by particle size measurements. As seen in Table 1, blank nanoparticles were smaller than drug loaded nanoparticles which can be attributed to the adsorption of drug to particle surfaces. These values were 175 nm and 184 nm for blank and etodolac loaded nanoparticles, respectively. Standard deviations of all formulations were very low, indicating unimodal monodisperse particle size distributions for blank and etodolac loaded nanoparticles. Etodolac loaded PLGA nanoparticles appear to be more advantageous regarding their potential of sterilization by membrane filtration due to their small size and favorable polydispersity index value.

Zeta Potential Measurement

Zeta potential values are also of interest for the characterization of the nanoparticles since blank and etodolac loaded nanoparticles display zeta potential values varying between -5 to -10 mV as seen in Table I. Zeta potential is a function of the surface charge of colloidal dispersions. It is commonly used to predict and control dispersion stability. Zeta potential data suggest the potential physical stability of nanoparticles in aqueous dispersion state.

TABLE I

Comparison of particle sizes and polydispersity indexes of blank and etodolac loaded nanoparticles

Formulations	Mean diameter (nm) ± S.D.	PI	Zeta potential (mV) ± S.D.
Blank nanoparticles	175 ± 11	0.04	-9.7 ± 1.9
Etodolac loaded nanoparticles	184 ± 1	0.03	-7.6 ± 1.5

Scanning Electron Microscope (SEM) Analysis

Imaging of nanoparticles by SEM is expected to provide information on nanoparticle morphology and size. Examination of SEM photographs of the nanoparticles revealed that the surfaces were smooth and spherical, as seen in Figure 4.

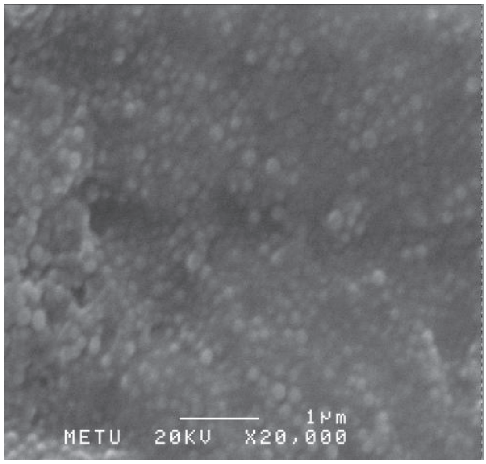

Figure 4

SEM of PLGA nanoparticles

Drug Loading and Yield

The yield of the drug loaded nanoparticle formulation was found to be between 80 and 89 % (82±3 %). Relatively higher yield was obtained with pluronic F68. The results showed that the encapsulation efficiency was about 16.5 %. Analytical method of etodolac quantification by UV spectrophotometry was validated (linearity r^2: 0.999) as seen Figure 5. In literature, the encapsulation efficiencies of the various nanoparticle formulations were reported about 10-40 %. Considering that a certain amount of drug may be lost during loading process[15,16].

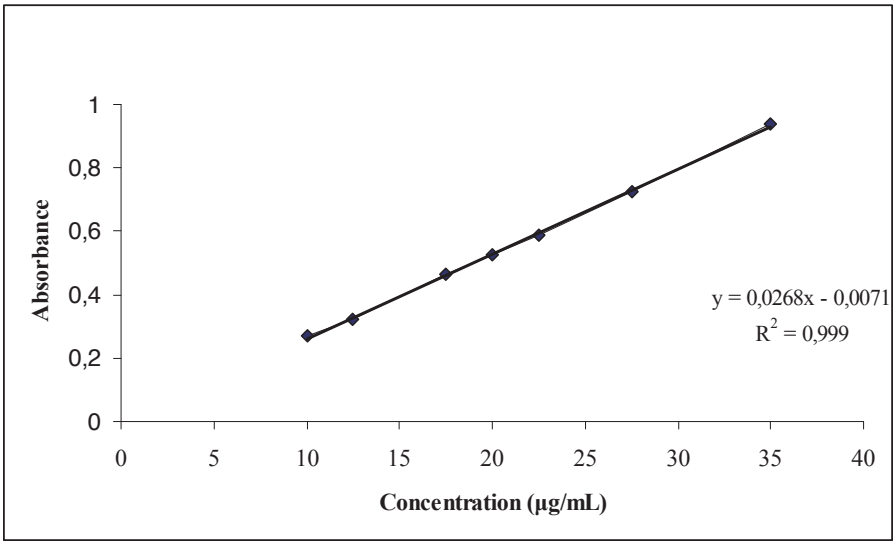

Figure 5

Calibration curve of etodolac in PBS:PEG_{400} (60:40)

In Vitro Drug Release

In vitro release of etodolac was assessed from nanoparticles into PBS (pH 7.4):PEG_{400} (60:40). The release profile of etodolac from PLGA nanoparticles is illustrated in Figure 6. It can be seen that nanoparticles liberate the drug in a considerably slower release profile. 50 % of the drug was released within 4 h. 70 % release of etodolac was realized within a period of up more than 72 h. The rapid burst effect is very much delayed with PLGA nanoparticles.

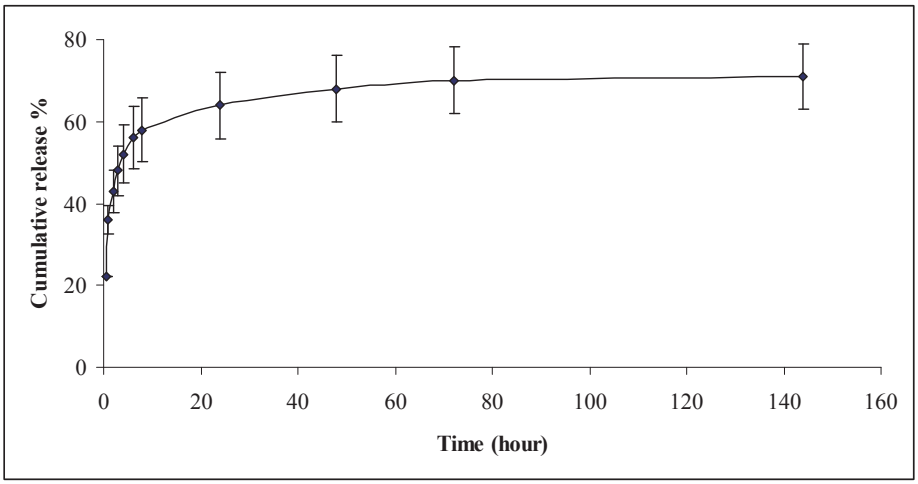

Figure 6

In vitro release study in PBS:PEG_{400} (n=6±S.D.)

In this study, a new nanoparticulate delivery system for etodolac has been designed and evaluated for in vitro properties. In the light of these findings, it can be concluded that etodolac loaded PLGA nanoparticles of appropriate particle size are obtained by nanoprecipitation technique. High drug loading and yield values were achieved. In vitro release data indicate that the drug is encapsulated in the membrane. This way it is possible to avoid burst effect and extended the release profile of nanoparticles to 144 h.

Summary

Etodolac is a nonsteroidal anti-inflammatory drug (NSAID) which blocks the enzyme that makes prostaglandins. NSAIDs treat the symptoms of pain and inflammation. Etodolac is used to treat pain or inflammation caused by arthritis or osteoarthritis. The commonest side effects during therapy are generally gastrointestinal disturbances and nanoparticulate drug delivery systems may reduce these side effects. In this study, nanoparticles were prepared with nanoprecipitation technique. Particle size and zeta potential measurements were performed by Malvern Zetasizer (Malvern Instruments, UK). Mean particle sizes measured were 175 nm and 184 nm for blank and etodolac loaded nanoparticles, respectively

and polydispersity indices (PI) were lower than 0.2 for all formulations. Zeta potential values were negative. Examination of photographs of the nanoparticles with Scanning Electron Microscope (Jeol-SEM ASID-10, Japan) revealed that the surface were smooth and spherical. The encapsulation efficiency value of the formulation was found to be 17 %. Nanoparticle formulation showed a significant controlled release profile extended up to 144 h. These results indicated that etodolac-loaded PLGA nanoparticles might provide a promising carrier system for the effective delivery of this anti-inflammatory drug.

Keywords: Etodolac; poly(lactide-co-glycolide); nanoparticle; *in vitro* characterization

Özet

Etodolak Yüklü Poli (Laktid-Ko-Glikolid) Nanopartikülleri: Formülasyon ve *İn Vitro* Karakter

Etodolak prostaglandin sentezini inhibe eden nonsteroidal antienflamatuar ilaçtır (NSAİİ). NSAİİ'lar ağrı ve inflamasyon semptomlarını tedavi ederler. Etodolak artrit ve osteoartritin neden olduğu ağrı veya inflamasyon tedavisinde kullanılır. Tedavi sırasındaki en önemli yan etkileri genellikle gastrontestinal rahatsızlıklardır ve nanopartiküler ilaç taşıyıcı sistemler bu yan etkileri azaltabilir. Bu çalışmada, nanopartiküller nanopresipitasyon tekniğine göre hazırlanmıştır. Partikül büyüklüklüğü ve zeta potansiyel ölçümleri Malvern Zetasizer (Malvern Instruments, UK) aleti ile yapılmıştır. Ortalama partikül büyüklükleri boş ve etodolak yüklü nanopartiküller için sırasıyla 175 ve 184 nm bulunmuştur ve bütün fomülasyonlar için polidispersite indeksleri (PI) 0.2'den küçüktür. Zeta potansiyel değerleri negatiftir. Taramalı elektron mikroskobu (Jeol-SEM ASID-10, Japan) ile incelenen nanopartikül fotoğraflarında yüzey düzgün ve küresel bulunmuştur. Formülasyonun ilaç yükleme değeri % 17 bulunmuştur. Nanopartikül formülasyonu 144 saate kadar uzayan kontrollü salım profili göstermiştir. Bu sonuçlar etodolak yüklü PLGA nanopartiküllerinin bu antiinflamatuvar ilaç için umut vaadedici taşıyıcı system olabileceğini göstermiştir.

Anahtar kelimeler: Etodolak; poli(laktid-ko-glikolid); nanopartikül; *in vitro* karakterizasyon

REFERENCES

1. Govender, T., Stolnik, S., Garnett, M. C., Illum, L., Davis, S.S.: PLGA nanoparticles prepared by nanoprecipitation: Drug loading and release studies of a water soluble drug, J. Controlled Release, 57, 171–185 (1999).
2. Breunig, M., Bauer, S., Goepferich, A.: Polymers and nanoparticles: Intelligent tools for intracellular targeting?, Eur. J. Pharm. Biopharm., 68, 112-128 (2008).
3. Rieux, A., Fievez, V., Garinot, M., Schneider, Y.J., Préat, V.: Nanoparticles as potential oral delivery systems of proteins and vaccines: a mechanism of approach, J. Controlled Release, 116, 1-27 (2006).
4. Raghavendra, C., Mundargi, C., Ramesh Babu, V., Rangaswamy, C., Patel, P., Aminabhavi, T.M.: Nano/micro technologies for delivering macromolecular therapeutics using poly(D,L-lactice-co-glycolide) and its derivatives, J. Controlled Rel., 125, 193-209 (2008).
5. Çırpanlı, Y., Ünlü, N., Çalış, S., Hıncal, A.A.: Formulation and in-vitro characterization of retinoic acid loaded poly (lactic-co-glycolic acid) microspheres, J. Microencapsulation, 22 (8), 877–889 (2005).
6. Barichello, J.M., Morishita, M., Takayama, K., Nagai, T.: Encapsulation of hydrophilic and liphophilic drugs in PLGA nanoparticles by the nanoprecipitation method, Drug Dev. Ind. Pharm., 25, 471-476 (1999).
7. Soppimath, K.S., Aminabhavi, T.M., Kulkarni, A.R., Rudzinski, W.E.: Biodegradable polymerics nanoparticles as drug delivery devices, J. Controlled Rel., 70, 1-20 (2001).
8. Kılıç; A.C., Çapan, Y., Vural, İ., Gürsoy, R.N., Dalkara, T., Cuine, A., Hıncal, A.A.: Preparation and characterization of PLGA nanospheres for the targeted delivery of NR2B-specific antisens oligonucloetides to the NMDA receptors in the brain, J. Microencapsulation, 22(6), 633-641 (2005).
9. Jain, R.A.: Manufacturing techniques of various drug loaded biodegradable poly (lactide-co-glycolide) (PLGA) devices, Biomaterials, 21, 2475-2490 (2000).
10. Taş, C., Özkan, Y., Okyar, A., Savaşer, A.: In vitro and ex vivo permeation studies of etodolac from hydrophilic gels and effect of terpenes as enhancers, Drug Deliv., 14(7), 453-459 (2007).
11. Barakat, N.S.: Etodolac-liquid-filled dispersion into hard gelatin capsules: an approach to improve dissolution and stability of etodolac formulation, Drug Dev. Ind. Pharm., 32 (7), 865-876 (2006).
12. Etodolac in the European Pharmacopéa vol 2, Strasbourg, 5th Ed, (2004), p.1560.
13. Fessi, H., Devissaguet, J.P., Thies, C.: Process for the preparation of dispersible colloidal systems of a substance in the form of nanospheres, US Patent, 5, 118,528 (1998).
14. Govender, T., Stolnik, S., Garnett, M. C., Illum, L., Davis, S.S.: PLGA nanoparticles prepared by nanoprecipitation: drug loading and release studies of a water soluble drug, J. Controlled Rel., 57, 171–185 (1999).
15. Kunii, R., Onishi, H., Machida, Y.: Preparation and antitumor characteristics of PLA/(PEG-PPG-PEG) nanoparticles loaded with camptothecin, Eur. J. Pharm. Sci., 67, 9-17 (2007).
16. Kunii, R., Onishi, H., Ueki, K., Koyama, K., Machida, Y.: Particle characteristics and biodistribution of camptothecin-loaded PLA/(PEG-PPG-PEG) nanoparticles, Drug Delivery, 15, 3–10 (2008).