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Development of nano-sized ketoprofen lysine incorporated Eudragit® S100 nanomedicine by double emulsion solvent evaporation and *in vitro* characterization

[Desarrollo de un nanofármaco de ketoprofeno lisina de tamaño nanométrico con Eudragit® S100 incorporado mediante doble evaporación del disolvente de emulsión y su caracterización *in vitro*]

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Abstract

Context: Pain has a very important effect on the biological, psychological, sociological and economic situation of a patient. Nanoparticles (NPs) are being extensively investigated as drug delivery systems worldwide for pharmaceutical applications.

Aims: To design and compare the release characteristics of sustained-release formulations of ketoprofen lysine (KL) NPs.

Methods: KL-Eudragit® S100 NPs were produced by double emulsion solvent evaporation method. The physicochemical characteristics of NPs were studied.

Results: Particle size of NPs prepared was in the range of 99 and 141 nm. Encapsulation efficiency (%) was obtained (76%) for NP formulations prepared. Weibull models were determined to be the most appropriate kinetic models for NP containing KL. KL-loaded NPs demonstrated nanostructural character and NPs showed extended release of KL.

Conclusions: NPs developed were found to be stable and representing a promising system for sustained delivery of KL.

Keywords: Eudragit® S100; ketoprofen lysine; nanoparticle.

Resumen

Contexto: El dolor tiene un efecto muy importante en la situación biológica, psicológica, sociológica y económica de un paciente. Las nanopartículas (NP) están siendo ampliamente investigadas como sistemas de administración de fármacos en todo el mundo para aplicaciones farmacéuticas.

Objetivos: Diseñar y comparar las características de liberación de formulaciones de liberación sostenida de NP de ketoprofeno lisina (KL).

Métodos: Las NP de KL-Eudragit® S100 se produjeron por el método de evaporación con doble emulsión de disolvente. Se estudiaron las características fisicoquímicas de las NP.

Resultados: El tamaño de partícula de las NP preparadas estaba en el intervalo de 99 y 141 nm. Se obtuvo la eficiencia de encapsulación (76%) para las formulaciones de NP preparadas. Se determinó que los modelos cinéticos de Weibull son los más apropiados para NP que contienen KL. Las NP cargadas con KL demostraron carácter nanoestructural y una liberación extendida de KL.

Conclusiones: Se encontró que las NP desarrolladas son estables y representan un sistema prometedor para la administración sostenida de KL.

Palabras Clave: Eudragit® S100; ketoprofeno lisina; nanopartícula.

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INTRODUCTION

Chronic pain is generally known as any type of pain that lasts longer than 12 weeks. Acute pain is a normal feeling that warns us against possible injuries, but chronic pain is much different. Chronic pain usually lasts for months or longer. Chronic regional pain is present in 20 to 25% of the population and chronic widespread pain is present in approximately 10% of the population (Crofford, 2015). Drugs used to treat chronic pain include antidepressants that increase synaptic norepinephrine and serotonin, neuronal excitability reducing agents and analgesics such as opioids and nonsteroidal anti-inflammatory drugs (NSAIDs) (Crofford, 2010). NSAIDs are among the most widely used drugs in the worldwide because of their efficacy in reducing pain and inflammation (Ong et al., 2007). The major therapeutic effects of NSAIDs are mainly accepted by their ability to block the synthesis of certain prostaglandins (PGs) by the inhibition of cyclooxygenase enzymes (COX-1 and COX-2). COX-1 produces PGs and thromboxane A_2 that control mucosal barrier in gastrointestinal tract, platelet aggregation, renal homeostasis and some physiological performance. On the other hand, COX-2 produces PGs that related to fever, inflammation and pain. The inhibition of COX-2 by NSAIDs probably represents the desired effect of the anti-inflammatory, antipyretic and analgesic response of NSAIDs; COX-1 inhibition plays an important role in adverse side effects such as GI and renal toxicity (Wongrakpanich et al., 2018).

Ketoprofen lysine (KL) is one of the most commonly used NSAIDs for the treatment of various chronic inflammatory diseases such as osteoarthritis and rheumatoid arthritis for analgesic activity. It is the water-soluble lysine salt of ketoprofen (Gentile et al., 1999; Cerciello et al., 2016). Compared with ketoprofen, KL showed better pharmacokinetic profile and excellent tolerability, increased absorption rate and improved gastric tolerance (Cimini et al., 2015). Besides these good features, it has a short half-life of 1 - 2 hours (Cerciello et al., 2016). In oral administration, multiple dose regimens are required, leading to fluctuations in drug blood levels and adverse effects due to toxicity, and sometimes inadequate treatment and poor patient compliance (Cerciello et al., 2016). Because of these features; KL is a candidate drug for prolonged-release dosage forms (Cerciello et al., 2016). Fig. 1 shows that chemical structure of KL.

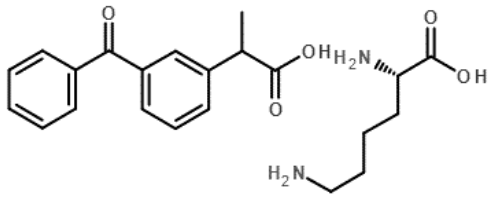

Figure 1. Chemical structure of ketoprofen lysine.

Nanoparticles (NPs) are matrix systems that are prepared with natural or synthetic polymers with sizes in the range of 10 to 1000 nanometer (nm). NPs, which attract attention of many research teams can be prepared by several methods. The methods used in nanoparticle preparation can be designed according to properties such as desired particle size and size distribution, etc. (Öztürk et al., 2017a). NPs are defined as either nanospheres or nanocapsules depending on their structures where the active substance is solubilized, entrapped and/or adsorbed onto the particle surface. Natural and synthetic polymers are used in the production of polymeric NPs. Both natural and synthetic particulate drug delivery systems are preferred to obtain controlled drug release for increasing the life quality of patients (Fu and Kao, 2010; Rao and Geckeler, 2011). A number of approaches can be used to manufacture NPs such as salting-out, solvent evaporation, supercritical fluid technology, micro-emulsion, mini-emulsion, surfactant-free emulsion, and interfacial polymerization (Re, 2006). Double emulsions are complex systems, also called "emulsions of emulsions", in which the droplets of the dispersed phase contain one or more types of smaller dispersed droplets themselves. Double emulsions have the potential of encapsulating both hydrophobic and hydro-

philic drugs, cosmetics, foods and other high value products. Techniques based on double emulsions are commonly used for the encapsulation of hydrophilic molecules, which suffer from low encapsulation efficiency because of rapid drug partitioning into the external aqueous phase in classical emulsions. In double emulsification technique, displacement of a lipophilic solvent with a water-miscible semi-polar solvent results in accumulation of polymer on the surface. Rapid diffuse of the nonsolvent reduces the interfacial tension between the two phases leading to an increase in surface area and thus formation of small organic solvent droplets. It is a very simple and economic method (Mishra et al., 2010; Iqbal et al., 2015). Eudragit® S100 is copolymer based on methacrylic acid and methyl methacrylate. It is generally used for the formulation of transdermal patches, NPs, microparticles, solid dispersions and spherical crystals. Eudragit® S100 has been used for various applications such as sustain release, bioavailability enhancement, improvement in micrometric properties (Patra et al., 2017).

This study aims to treat chronic pain effectively by low dose of KL in a prolonged release pattern. The need of design and producing a new prolonged-release dosage form containing KL is the major aim of the study with a small amount of active ingredient and low side effects.

MATERIAL AND METHODS

Materials

KL was kind gift from Berko İlaç (İstanbul/Turkey). Poly(methacylic acid-co-methyl methacrylate) 1:2 (Eudragit® S100) was obtained from Evonik (Germany). Polyethylene glycol sorbitan monooleate (Tween® 80) was the surfactant, purchased from Merck (Germany). Deionized and filtered water was used in all experiments (Milli-Q Academic, Millipore, Molsheim, France). All other chemicals were analytical grade.

Preparation of NPs

Eudragit® S100 NPs were prepared by 'Double Emulsification Solvent Evaporation Technique'. Firstly, weighed amount of Eudragit® S100 (0.3 g) was dissolved in 5 mL methanol. One mL aqueous solution of Tween® 80 (1% w/v) was added drop-wise to 5 mL Eudragit® S100 solution using ultrasonic bath (Wisd Laboratory Instruments, WUC-A03H, Korea) (1 min) (W_1/O). 5 mL of this emulsion was then added drop-wise to 20 mL of Tween® 80 aqueous solution (1%, w/v) using high-speed homogenizer (Ultraturrax T25, Janke & Kunkel IKA® Labortechnik, Staufen, Germany) with 13,000 rpm stirring for 2 min ($W_1/O/W_2$). Methanol was evaporated at room temperature in 4 hr to obtain an aqueous dispersion which was finally centrifuged (10,000 rpm, 45 min, 4°C) to collect the NPs (Rotina 420R, Hettich Zentrifugen, Germany).

For the preparation of KL-loaded Eudragit® S100 NPs, the procedure started by adding 0.03 g KL [10% KL (I-KL)] to 1 mL Tween® 80 aqueous solution (1% w/v). Tween® 80 aqueous solution containing KL was added drop-wise to 5 mL Eudragit® S100 solution using ultrasonic bath (Wisd Laboratory Instruments, WUC-A03H, Korea) (1 min) (W_1/O). A total of 5 mL of this emulsion was then added drop-wise to 20 mL of Tween® 80 aqueous solution (1%, w/v) under high-speed homogenizer (Ultraturrax T25, Janke & Kunkel IKA® Labortechnik, Staufen, Germany) with 13.000 rpm stirring for 2 min ($W_1/O/W_2$). Methanol was evaporated at room temperature in 4 h to obtain an aqueous dispersion, which was finally centrifuged (10,000 rpm, 45 min, 4°C) to collect the NPs (Rotina 420R, Hettich Zentrifugen, Germany).

Characterization of NPs

Particle Size, polydispersity index (PDI) and zeta potential

The particle size (PS) and polydispersity index (PDI) were measured using dynamic light scattering technique (DLS) on the Zetasizer Nano (Zetasizer Nano ZS, Malvern Instruments, Malvern, UK). PS and PDI of NPs prepared were measured by dispersing the formulation in distilled water. Zeta potential values were determined using the same instrument in a disposable folded capillary zeta cell, at 25°C room temperature and diluted with distilled water. For statistical

analysis all samples were measured in triplicate and the average values and standard deviation of the measurements were calculated.

Thermal analysis (DSC)

Physical state of KL incorporated was characterized by was characterized by differential scanning calorimetry (DSC) (DSC-60, Shimadzu Scientific Instruments, Columbia, MI, USA). Aluminum crucibles with 5 mg samples were analyzed under nitrogen gas (50 mL/min) and heating rate of 10°C/min at a temperature range of 30 and 300°C. Pure KL, pure Eudragit® S100, physical mixture and blank formulation were also analyzed.

X-ray diffraction (XRD) analysis

XRD analyses were performed (Rikagu Corporation D/Max-3C, Japan) within the range of 5-55° at 2θ with 2°/min scanning rate and using 40 kV voltage with 20 mA current intensity level. XRD spectra were also obtained for pure Eudragit® S100, pure KL, physical mixture and blank formulation were used as references.

Infrared (FT-IR) analysis

FT-IR spectra were recorded using Shimadzu IR Prestige-21 (Shimadzu Corporation, Kyoto, Japan) at the wavelength range of 4000-500 cm^{-1}. Pure Eudragit® S100, pure KL, physical mixture and blank formulation were also analyzed and were used as references.

High performance liquid chromatography (HPLC)

Entrapment efficiency (EE %) and dissolution of each formulation was tested using HPLC (Shimadzu Corporation-20A, Kyoto, Japan) with reversed-phase InertSustaine® column (4.6 mm*150 mm, C_{18} Gravity, 5 µm) or the determination of KL using a modified method. Mobile phase of the HPLC system was a mixture of acetonitrile:0.03 M KH_2PO_4 buffer (60:40, v/v) (pH value 3.0 was adjusted with orthophosphoric acid) prepared daily, degassed by sonication and filtered through 0.45 µm membrane filter just before testing. Flow rate was set at 1 mL/min resulting in a run time of 15 min per sample and injection volume was 20 µL. A wavelength of 242 nm was used for detection and samples were analyzed at 40°C. HPLC method used was validated for linearity, precision, accuracy and specificity (Anacardio et al., 2008; Öztürk et al., 2017b).

Entrapment efficiency (EE %)

KL loading to Eudragit® S100 NPs was determined by HPLC method described in the previous section. KL, the active agent of Eudragit® S100 NPs was evaluated by coordinate extraction of KL from NPs. Lyophilize NPs (5 mg) were precisely measured, 1 mL methanol was added and vortexed to break up the particles in the natural stage. Complete solution was filtered through 0.22 µm polyamide filter and analyzed using HPLC. Drug content was expressed as EE (%) following Equation 1 (Martín-Banderas et al., 2012).

EE % = (Actual amount of KL loaded in Eudragit® S100 NPs/Theoretical amount of KL loaded in Eudragit® S100 NPs) X 100 [1]

Dissolution

In vitro release of KL from Eudragit® S100 NPs was investigated over 48 h using a dialysis membrane. NP containing 5 mg KL was placed in a cellulose acetate dialysis bag (dialysis tubing cellulose membrane average flat width 33 mm [1.3 in.], molecular weight cut-off [MWCO]:14,000, D9652, Sigma-Aldrich®,USA). After the addition of 1 mL of dissolution medium, the bag was sealed at both ends. Dialysis bag was then placed into an amber glass beaker containing 100 mL PBS (pH 7.4, Solution prepared with phosphate buffered saline tablet, P4417, Sigma-Aldrich®,USA) at 37 ± 1°C as the dissolution medium under continuous stirring of 100 rpm (MS-33MH Magnetic stirrer, Jeiotech, Korea). The receptor compartment was closed to prevent evaporation of the dissolution medium. Samples were taken at regular time intervals to be 1 mL and the same volume was replaced with fresh dissolution medium. KL concentration in the samples was quantified by HPLC method.

Kinetic analysis of dissolution data

Data obtained in the *in vitro* drug release studies was further investigated for release kinetics using DDSolver software program (Zhang et al. 2010).

RESULTS AND DISCUSSION

Particle size, polydispersity index (PDI) and zeta potential

Characterizations of NPs are primarily evaluated by the particle size (PS) and size distribution (PDI). Application of NPs in drug targeting and drug release can be conveniently determined by various tools. It has already been reported that particle size of nanoparticles has profound effect on the drug release. According to the research, the fastest and most popular method for determining PS and PDI are preferred. The fastest and most popular techniques such as photon-correlation spectroscopy (PCS) or dynamic light scattering (DLS) are widely used to determine the size of Brownian nanoparticles in colloidal suspension at nano and micron intervals (de Assis et al., 2018). DLS is preferred in this work because it is the most commonly used technique for accurate estimation of PS and PDI (Öztürk et al., 2017a). The results in Table 1 shows the PS and PDI values of NPs. The PS obtained in the I-Blank coded placebo formulation was observed to be 99.07 ± 2.69 nm while the particle size in the formula I-KL containing KL was determined to be 141.77 ± 1.85 nm. It was observed that the particle size of the formulation prepared with the active ingredient increased compared to the placebo formulation. Previous studies have shown that active substance loaded NPs have a larger particle size than placebo NPs (Suhaimi et al., 2015). PDI value which defines size distribution is in the range of 0.01 and 0.5 - 0.7 for monophasic systems; value higher than 0.7 are indicative of a very wide size distribution and the value close to zero means narrow size distribution (Öztürk et al., 2018a). When the PDI results in Table 1 are examined, it is observed to be less than 0.5. Therefore, the distribution of the NPs prepared in this study is monodisperse. Surface charge and intensity determines the interaction of nanoparticles with the biological environment as well as their electrostatic interaction with bioactive compounds. Stability of colloidal material is usually analyzed through zeta potential (ZP) of NPs. ZP is an indirect measure of the surface charge. It can be obtained by evaluating the potential difference between the outer Helmholtz plane and the surface of shear. Thus, ZP of colloidal based dispersion assists in directly evaluating its storage stability. ZP values (high zeta potential values, either positive or negative) are achieved in order to ensure stability and avoid aggregation of the particles (Pangi et al., 2003). The ZP values of the NPs prepared, measured in water, are presented in Table 1. All results are given by standard deviation (± SD). The mean ZP of all formulations ranged from 16.43 ± 1.27 mV to 24.33 ± 0.40 mV, which may be attributed to the positive charges on polymer matrices indicating good physical stability. Cationic property of NPs was determined due to the effect of positively charged quaternary ammonium groups in Eudragit® S100 interact with the negatively charged mucus and open up the tight junctions of epithelial cells to allow the paracellular transport pathway resulting in an increase in bioavailability (Yenilmez, 2017).

Table 1. Results of PS, PDI, ZP and EE%.

Code	PS (nm)	PDI	ZP (mV)	EE%
I-Blank	99.07 ± 2.69	0.13 ± 0.05	+24.33 ± 0.40	-
I-KL	141.77 ± 1.85	0.47 ± 0.02	+16.43 ± 1.27	76.54 ± 2.79

PS*: Particle size, PDI*: Polydispersity index, ZP*: Zeta potential, EE%*: Encapsulation efficiency (%), Mean ± Standard deviation.

Thermal analysis (DSC)

The thermal properties of the pure KL, pure Eudragit® S100, physical mixtures of KL with Eudragit® S100 and NPs were characterized by DSC analysis. DSC was used to detect the effect of molecular structure of the used polymer on the thermal properties of NPs. Moreover, DSC was also used for detecting the state of the encapsulated drug inside the NPs matrix as well as investigating any possible interaction between the drug and the polymeric matrix (Öztürk et al., 2017a; Yenilmez, 2017). DSC thermograms of all freshly prepared NPs are given in Fig. 2 in comparison to KL, Eudragit® S100 and the physical mixture. DSC thermogram of pure KL showed a single endothermic peak at 170.09°C, related to the drug's melting point. In addition, endothermic peak of KL at 170.09ºC was compatible with the literature (Cerciello et al., 2016). No clear peak presented in the thermograms of the prepared NPs due to any possible decrease in the drug crystallinity and/or solvation of the drug in the melted carrier and/or heat-induced interaction between drug and polymer. Such findings have been reported for Eudragit® based NPs (Jafari-Aghdam et al., 2016; Öztürk et al., 2017a).

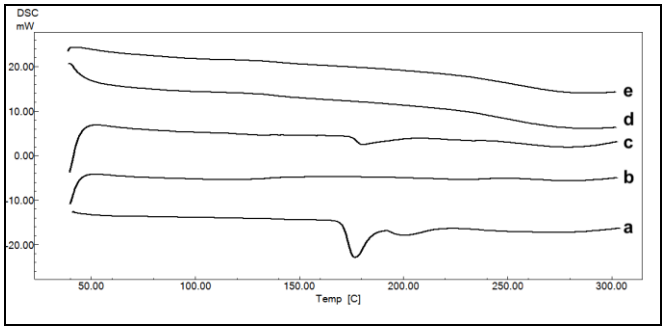

Figure 2. DSC analysis results.

a: Ketoprofen lysine (KL); **b:** Eudragit® S100; **c:** Physical Mixture; **d:** I-Blank **e:** I-KL.

X-ray diffraction (XRD) analysis

XRD analysis is a fundamental and important technique in the pharmaceutical field. It plays a major role in drug development, testing and production (Chauhan and Chauhan, 2014). XRD analysis is a well-defined analytical method frequently used in research because it reveals the molecular structure of NPs, examines the crystal state, performs polymorphism studies and also provides information about stability. XRD analysis is based on determining the diffraction pattern of the X-rays from a sample as a function of scattering angle. It is used to investigate the crystalline properties of drugs (Lin et al., 2014; Öztürk et al., 2018c). XRD profiles of pure KL, pure Eudragit® S100, physical mixtures of KL with Eudragit® S100 and NPs prepared are shown in Fig. 3**.**

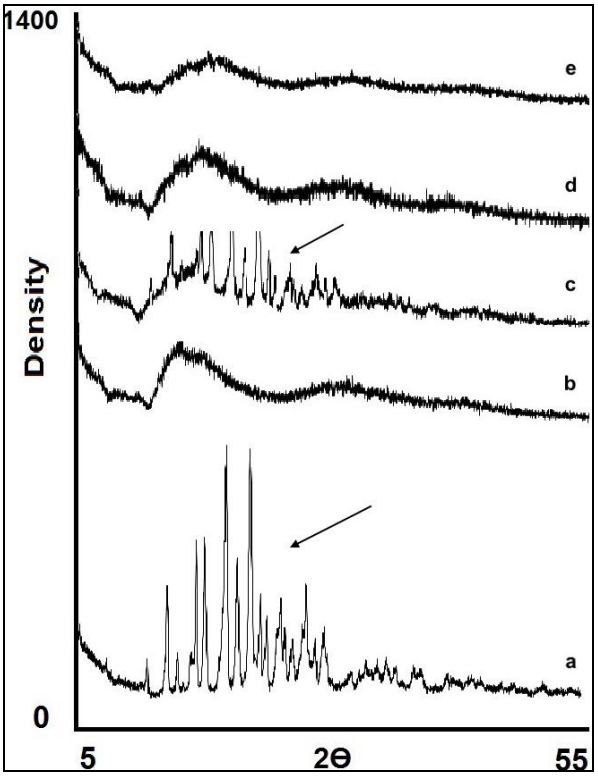

Figure 3. XRD analysis results.

a: Ketoprofen lysine (KL); **b:** Eudragit® S100; **c:** Physical mixture; **d:** I-Blank; **e:** I-KL.

This figure clearly shows that KL exhibits crystal structure while the X-ray diffractogram of Eudragit® S100 is typical of amorphous structure. I-KL coded NPs formulation showed that typical KL peak intensity was lowered due to the dilutional effect exerted by the Eudragit® S100 polymer network but with no qualitative variation in KL diffractogram. This decrease in KL crystallinity in the formulation affirmed drug amorphization and

subsequent internalization into the polymeric system. Characteristic peaks of KL were not observed in XRD profiles of I-KL coded NPs formulation. This indicates that KL was molecularly dispersed within NPs and there exists less or no free drug in crystalline form on the surface of nanoparticles (Kırımlıoğlu et al., 2016). Absence of the drug melting peak and diffraction peaks of the crystal structure of the drug in DSC thermogram and XRD pattern, respectively, are usually signs of amorphous or solid solution state of the drug within the polymer.

Infrared (FT-IR) analysis

FT-IR spectra of all freshly prepared NPs are given in Fig. 4 in comparison to KL, Eudragit® S100 and the physical mixture. FT-IR can be used for identifying any chemical interaction between the drug and the polymer. A FT-IR vibrational spectrum, characteristics for a given structure, is usually obtained. Any reduction or disappearance of the FT-IR characteristics peaks of the encapsulated drug indicates an interaction between the drug and the polymer might have occurred spectrum shows absorption bands or C=O stretching vibration of acid, C=O tretching vibration of ketone, O-H band and C=C tretching vibration of the aromatic ring appeared at 664.57, 1631, 3300-3100 cm^{-1}, respectively. Bands at 1587.42 and 1066.00 - 1138.00 cm^{-1} were assigned to N–H in-plane bending and C–N stretching of amine, respectively (Carneiro et al., 2016). Spectrum of Eudragit® S100 showed stretching of carboxylate anion at 1386.82 cm^{-1}, superimposed C=O stretching of alkyl ester and carboxylic acid at 1724.36 cm^{-1}, C–(C=O)–O and O–C–C stretching around at 1149.57 cm^{-1} (Ahuja et al., 2011). The main difference between KL and Eudragit® S100 is the C–N stretching of amine (1066 cm^{-1}). C–N stretching were observed prominently in KL, Physical mixture, I-KL; but were not observed in Eudragit® S100 and I-Blank. This indicated that KL is molecularly dispersed and encapsulated in the polymeric structure supporting DSC and XRD results (Öztürk et al., 2017a; Öztürk et al., 2018c).

High performance liquid chromatography (HPLC)

HPLC method was validated for precision, accuracy, specificity and linearity. Linearity was determined to be at the concentration range of 10 - 70 µg/mL. The method developed for KL was decided to be precise due to RSD values of <2 % for repeatability and intermediate precision. Recovery of the method was satisfactory owing to <2 % RSD value. Conclusively, procedure proposed in this study can be used for routine, simultaneous and concurrent KL determination. Validation of the HPLC method used for the determination of KL was determined using 7 different concentrations (from 10 µg/mL to 70 µg/mL) and KL showed a linearity of y = 525398x-56802 (r^2= 0.9999) linearity, accuracy of 99.608 ± 0.495%, 99.966 ± 0.107% and 100.112 ± 0.134% for the concentrations of 15, 30 and 45 µg/mL, respectively (n= 6). Limit of detection (LOD) was determined to be 0.0283 µg/mL while limit of quantitation (LOQ) was 0.0858 µg/mL. The proposed procedure can be used for routine, simultaneous and concurrent determination of KL (Anacardio et al., 2008; Öztürk et al., 2017b; Öztürk et al., 2018b).

Entrapment efficiency (EE %)

Nanoparticles composed of natural/synthetic polymers or lipids are usually smaller than 1000 µm in size. Active drug ingredient may either be incorporated into the matrix or superficially adsorbed. The classical utilized double emulsion solvent evaporation technique for encapsulating water-soluble KL in Eudragit® S100 nanoparticles suffers from low encapsulation efficiency because of the drug rapid partitioning to the external aqueous phase. EE % values calculated according to Eq. 1 are given in Table 1. Entrapment efficiency of NPs is an important factor in formulations because higher loading requires smaller amounts of NPs for a given dose (Martín-Banderas et al., 2012). The NP, made of Eudragit® S100, were prepared by the double emulsion solvent evaporation technique and showed high EE. EE% was found to be 76.54% ± 2.79. High EE will enable the patient suffering from chronic pain to use less weight nanoparticles in the drug regimen.

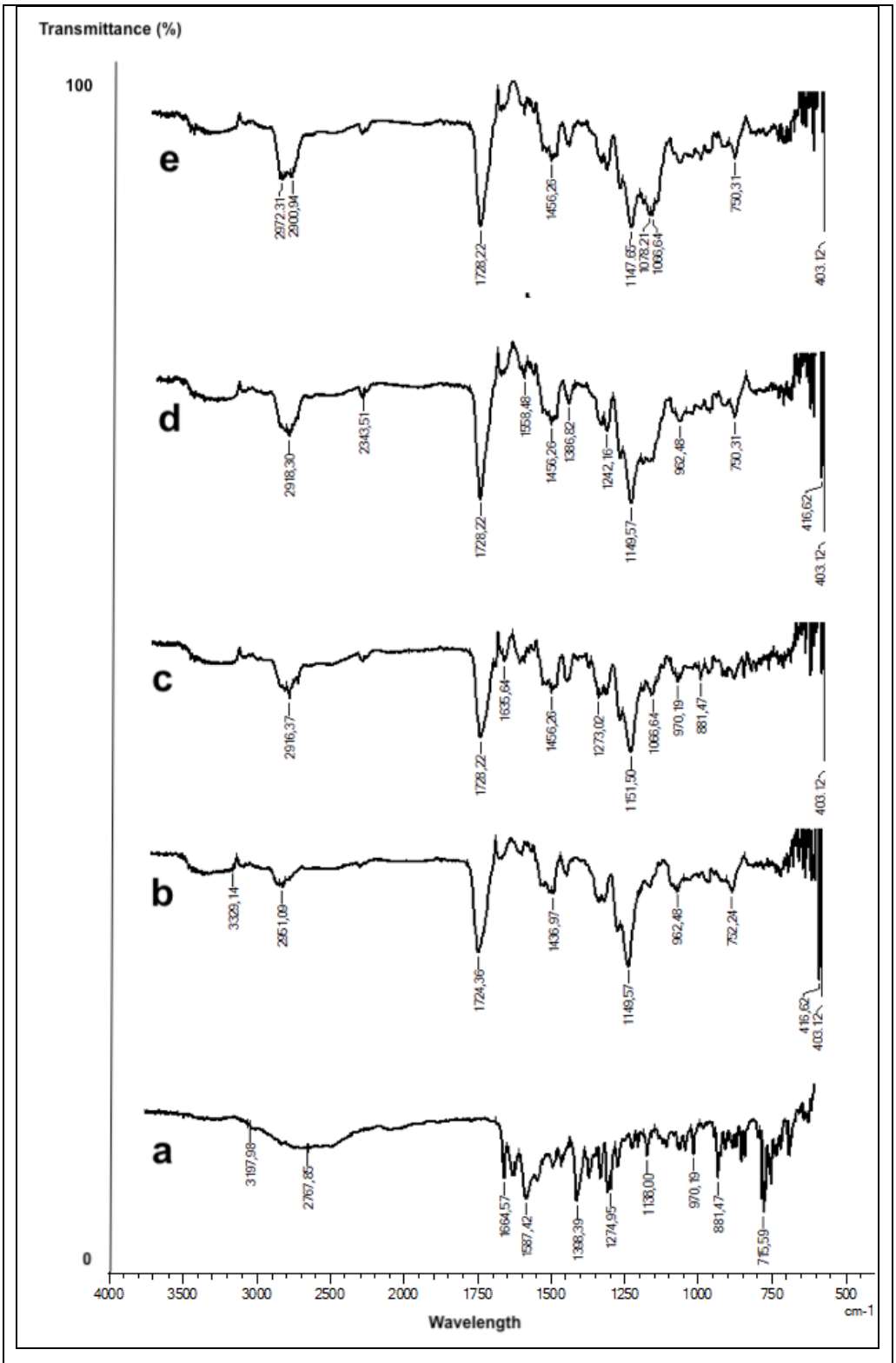

Figure 4. FT-IR analysis results.

a: Ketoprofen lysine (KL); **b:** Eudragit® S100; **c:** Physical mixture; **d:** I-Blank; **e:** I-KL.

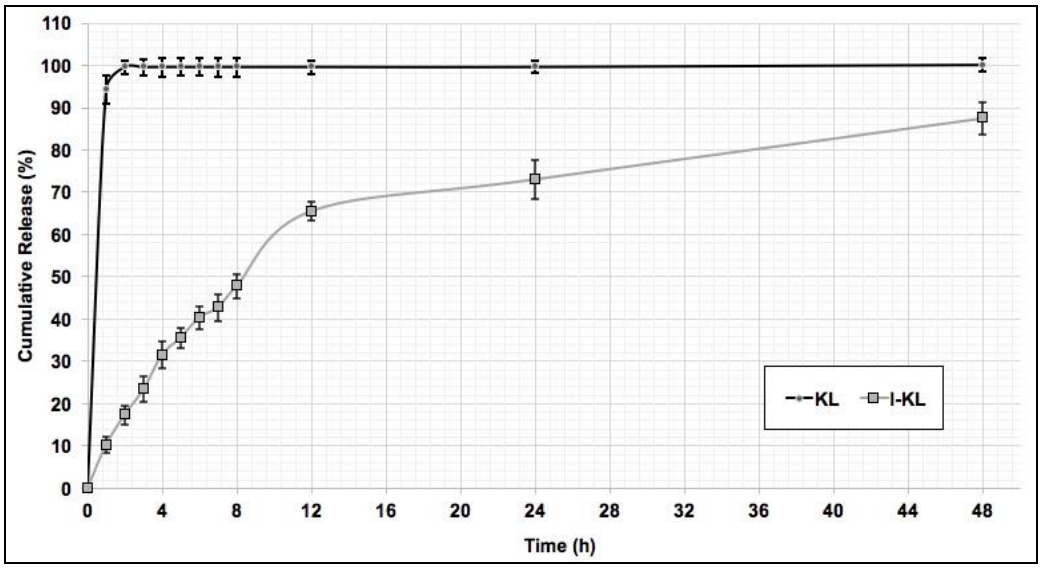

Figure 5. *In vitro* dissolution profile of pure ketoprofen lysine (KL) and KL-loaded Eudragit® S 100 NP (I-KL).

Dissolution

In vitro release test results are frequently used not only for monitoring release profiles of drugs but also for predicting in vivo absorption (Zhang et al., 2010). The *in vitro* release profiles of KL from the I-KL coded NP shown in Fig. 5. The amount of KL released from the pure KL at pH 7.4 medium is 94.40 ± 3.3% at 1 h, whereas that of KL released from the I-KL coded NP Formulation is no more than 10.20 ± 2.1%. Release of KL from NP was observed to show a biphasic profile consisting of a rapid initial phase followed by a slow release phase which lasted over 48-hours. Drug release during sustained release phase is mainly controlled by solubility of the drug in the matrix, diffusion of drug into the matrix, and matrix erosion. Due to the short half-life of KL and rapid release from the conventional tablet formulations marketed, patients need to administer the drug at least 2-3 times a day. Therefore, preparing NP to provide initial dose with the superficial KL and maintenance dose with KL entrapped was aimed in this study. Initial rapid release observed from NP was most probably dependent on the rapid dissolution of superficially adsorbed KL and it was found that KL entrapped in NP was released in a sustained pattern (Öztürk et al., 2017a).

Determination of *in vitro* kinetics with DDSolver program

When a new oral dosage form is developed, the dissolution behavior should be quantitatively analyzed. Dissolution analysis involves the comparison of dissolution profiles and the application of mathematical models to identify the drug release pattern. In this study, DDSolver named application, which is an Excel add-on software package designed to analyze the data obtained from dissolution experiments, is used (Zuo et al., 2014). The selection of a suitable model for fitting dissolution data is essential, not only for quantitative evaluation of drug release characteristics but also for comparison of dissolution profiles using model-dependent approaches (Zhang et al., 2010; Öztürk et al., 2017a; Öztürk et al., 2018a). The DDSolver provides a number of statistical criteria for evaluating the conformance of a model, including the correlation coefficient (R_obs-pre), the coefficient of determination (Rsqr, R^2, COD), the adjusted coefficient of determination corrected (Rsqr_adj, $R^2_{adjusted}$), mean square error (MSE), standard devi-

ation of the residuals (MSE_root or Sy.x), sum of squares (SS), Weighted sum of squares (WSS), Akaike Information Criterion (AIC) and Model Selection Criteria (MSC) (Zhang et al., 2010). Among these criteria, $R^2_{adjusted}$, AIC and MSC are the most popular in the resolution model definition (Zhang et al., 2010). In this study, Zero-order kinetics, First-order kinetics, Higuchi, Korsmeyer-Peppas, Hopfenberg, Hixon-Crowell, Baker-Lonsdale and Weibull models were selected for evaluation in DDSolver program. After calculation of cumulative amount released, data obtained was transferred to DDSolver program to determine important criteria, $R^2_{adjusted}$, AIC, MSC. The highest $R^2_{adjusted}$ and MSC values and the lowest AIC values are used for the evaluation of the best fit. When examined Table 2 and comparing those models according to the important criteria mentioned above, Weibull model was selected to be the best kinetic model. The release profiles were fitted to Weibull, indicating diffusion mechanism which was expected to control the drug release. In Weibull model, it is considered that some of the factors affecting the overall drug release e.g. effective surface area are rarely mass dependent, mass of unreleased drug in the medium, while the others are supposed to be time dependent (Soltani et al., 2016). This result was also supported by previous studies on Eudragit® NPs displaying that drug release is always the result of both dissolutive and diffusional phenomena (Soltani et al., 2016; El-Nahas et al., 2017).

CONCLUSIONS

In this study KL incorporated Eudragit® S100 NPs were formulated for extended analgesic delivery for oral application. *In vitro* characteristic properties of the polymeric NPs were evaluated in detail. Particle size of NPs prepared was in the range of 99 and 141 nm. Analysis results demonstrated that nano-sized particles prepared with double emulsion solvent evaporation method. In solid state characterization (DSC, XRD, FT-IR and NMR) results supported each other and these results proved that KL was loaded into the polymer. Encapsulation efficiency (%) was obtained (76%) for NP formulations prepared. Due to the high encapsulation efficacy of the nanoparticles, KL release could be extended up to 48 hours which will enhance the therapeutic efficacy of the formulations prepared. Therefore, analyses results can be concluded as KL loaded Eudragit® S100 NPs are effective carrier candidates for the oral application of KL for extended release.

Table 2. *In vitro* kinetic data.

Model	Equation	R^2	$R^2_{adjusted}$	MSE	MSE_root	SS	WSS	*AIC*	*MSC*
Zero-order	$F = k_0 * t$	-0.051	-0.051	600.881	24.513	6008.807	6008.807	97.711	-0.231
First-order	$F = 100 * [1-e^{(-k1*t)}]$	0.938	0.938	35.413	5.951	354.134	354.134	66.566	2.600
Higuchi	$F = k_H * t^{0.5}$	0.905	0.905	54.149	7.359	541.487	541.487	71.238	2.176
Korsmeyer-Peppas	$F = k_{KP} * t^n$	0.802	0.780	126.087	11.229	1134.780	1134.780	81.376	1.254
Hixson-Crowell	$F = 100 * [1-(1-k_{HC}*t)^3]$	0.837	0.887	93.377	9.661	933.367	933.367	77.227	1.631
Hopfenberg	$F = 100 * [1-(1-k_{HB}*t)^n]$	0.838	0.820	102.976	10.148	926.781	926.781	79.149	1.456
Baker-Lonsdale	$3/2 * [1-(1-F/100)^{2/3}]-F/100 = k_{BL}* t$	0.950	0.950	28.832	5.370	288.318	288.318	64.305	2.806
Weibull	$F = 100 * [1 - e^{-(t-Ti)\beta/a}]$	0.984	**0.980**	11.522	3.394	92.178	92.178	**55.761**	**3.582**

*In all models, R^2: coefficient of determination, R^2adjusted: adjusted coefficient of determination corrected, MSE: mean square error, MSE_root: standard deviation of the residuals, SS: sum of squares, WSS: Weighted sum of squares, AIC: Akaike Information Criterion, MSC: Model Selection Criteria. F is the fraction (%) of drug released in time t, k0: zero-order release constant, k1:first-order release constant, kH: Higuchi release constant, kKP: release constant incorporating structural and geometric characteristics of the drug-dosage form, n: is the diffusional exponent indicating the drug-release mechanism, kHC: Hixson-Crowell release constant, kHB: Hopfenberg release constant, kBL: Baker-Londsdale release constant, F0 is the initial fraction of the drug in the solution resulting from a burst release, α: is the scale parameter which defines the time scale of the process; β: is the shape parameter which characterizes the curve as either exponential (β=1; case 1), sigmoid, S-shaped, with upward curvature followed by a turning point (β>1; case 2), or parabolic, with a higher initial slope and after that consistent with the exponential (β<1; case 3), Ti: is the location parameter which represents the lag time before the onset of the dissolution or release process and in most cases will be near zero.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Contribution	Öztürk AA	Çinar NI	Yenilmez E
Concepts or ideas	x		
Design	x		
Definition of intellectual content	x		x
Literature search	x	x	x
Experimental studies	x	x	x
Data acquisition	x	x	x
Data analysis	x	x	x
Statistical analysis	x		x
Manuscript preparation	x		
Manuscript editing	x		
Manuscript review	x	x	x

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