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Thermosensitive pluronic® F127-based in situ gel formulation containing nanoparticles for the sustained delivery of paclitaxel

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Abstract

Bone metastasis is one of the most encountered complications among cancer patients and majority of cancer types has led to bone metastasis. Paclitaxel (PCX) is an anticancer agent commonly used in cancer treatment. However, its clinical use is restricted owing to poor water solubility. PCL NPs were investigated to cope with solubility problem of PCX. The size, polydispersity index and zeta potential of PCL were 383.8±2.4 nm, 0.253±0.122 and +51.3±6.1 mV, respectively. The PCX encapsulation efficiency was 77.2±2.1%. Subsequently, in situ gellling system was prepared by using different Pluronic F-127 concentration in order to determine the optimum ratio. İn situ gel formulation containing 20% Pluronic F-127 was selected as the optimum formulation and subjected to characterization tests. The viscosity of in situ gelling system with CS/PCX-PCL NPs at room temperature (25 °C±0.1) and at body temperature (37 °C±0.1) were found 137.00 ±3.05 cP and 890.30 ±89.61 cP at 100 rpm, respectively. According to the release results, in situ gel provided prolonged release profile compared to PCL NPs alone. Consequently, in situ gel containing CS/PCX-PCL NP elucidated in detail is a promising approach for locally applicable injectable systems.

Keywords: Cancer, nanoparticles, paclitaxel, in situ gel, drug release

Introduction

Bone metastasis is a secondary complication caused by the spread of cancer to the bone. Up to 70% of people with breast cancer or prostate cancer, and 15% to 30% of those with lung, colon, bladder, or kidney cancer, encounter bone metastases [1, 2]. Paclitaxel (PCX) is an anti-tumor agent which used in the treatment various cancers including bone metastasis [3, 4]. PCX exerts its therapeutic effect by targeting microtubules during cell profileration and inhibits cells at the G2/M phase [5]. Although PCX has been accepted as an ideal option in current clinical treatments, PCX shows a broad distribution throughout the body resulting in severe adverse effects [6]. Therefore, local applicable methods stand out to decrease severe side effects of PCX. Intraarticular delivery offers many advantages for joint and cartilage related diseases because only a small amount of the drug is required to exhibit the desired therapeutic efficacy, and drug exposure to improper areas is minimized [7].

Drug delivery systems (micro/nanoparticles, liposomes, and gels) for intraarticular injection can be effective to extend release period and retention time and to target specific sites like synovial joints, cartilage, chondrocytes, to improve the efficacy of drugs [8]. Polymeric nanoparticles (NPs) are drug delivery systems in the range of 10 to 1000 nm which consisted of biocompatible polymers [9, 10]. Polycaprolactone (PCL) is a biodegradable

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semicrystalline aliphatic polyester approved by the FDA that is used as a carrier for sustained delivery of therapeutic molecules [11]. Since PCL has slow in vivo degradation, non-toxic structure and therapeutic activity and stability, it is commonly preferred to fabrication of nanoparticle systems. Application of PCL NPs with in situ gelling system for local delivery of therapeutic molecules provides NPs with extended release at the site of action [12]. Chitosan (CS) is a deacetylated derivative of chitin. It is biodegradable and biocompatible cationic polymer and has benefits on bone regeneration [13]. It has been shown that chitosan prevents the formation of secondary breast and prostate tumors in the bone [14]. Furthermore, CS has been widely used as coating material. Surface decoration with CS provides a wealth of benefits for polymeric, lipid or metallic NPs, such as improving stability, enhanced bioavailability, controlled drug release, increased bioadhesion and increased cellular uptake [15].

Because of their unique characteristics, in situ gelling hydrogel formulations have gained great interest for development of site spesific drug systems. The hydrophilic nature of hydrogels limits the delivery of hydrophobic drugs in hydrogels. To overcome this problem, before being incorporated in hydrogels, hydrophobic active substances can be encapsulated in nanomaterials or their hydrophilicity can be increased with cyclodextrin derivatives [16]. The main limitations of thermosensitive hydrogels are in the development of biocompatible and biodegradable materials with ideal properties such as heat responsiveness and controlled/ sustained release. However, increasing researches have been reported due to the advances observed in the fields of drug delivery systems, biocompatible polymers and new polymeric drug formulation approaches for biomedical applications [17-19].

The simple phase transition (sol-gel transition) in water provides simplicity and safety as there are no chemical reactions or external stimuli that form the hydrogel. The sol phase is defined as a flowing fluid, whereas the gel phase is non-flowing while retaining its integrity. The gel phase appears when a polymer's critical concentration (critical gel concentration, CGC) is exceeded [20].

Various polymers can be used for the preparation of in situ gelling systems. Among these, poloxamers are popular polymers in pharmaceutical applications due to their favorable properties such as non-toxic, non-irritant, commercial availability and a wide variety of molecular weight forms. Poloxamers are amphiphilic synthetic tri-block copolymers comprised of two poly-(ethylene oxide) units and one poly(propylene oxide) (PPO) unit. They have been approved by the FDA and are listed in the US and European Pharmacopoeia [21].

Poloxamer 407 (Pluronic F127) gels at a concentration of 20% by weight at 25°C. This rate is lower than other members of the poloxamer series. Poloxamer gels are a mobile viscous liquid that, at room temperature, transforms into a semi-solid transparent gel at body temperature from the solution (37°C). At the crucial temperature for micellization, PPO block dehydration results in the production of micelles. As the temperature rises, micelle formation becomes increasingly crucial, and eventually the micelles come into contact and stop moving [13].

In our previous study, PCX loaded PCL NPs coated with CS (CS/PCX-PCL) and poly-L-lysine were developed. According to the results of in vitro characterization and in vitro release studies, CS coated formulations exhibited ideal properties in terms of particle size, zeta potential and poly dispersity index [22]. In this study, Pluronic F-127 (PF127) based thermosensitive in situ gel systems were developed. The gelation characteristics of each polymer and their formulations were investigated in order to determine the best conditions for forming in situ gelling systems. Then, CS/PCX-PCL NPs were dispersed in optimized thermosensitive in situ gel. This research lays the groundwork for the development of a PCX delivery system that combines the in situ thermogelling property of F127 with the benefits of polymeric NPs.

Material and Methods

Polycaprolactone (PCL) (Mw:80.000), Pluronic F-127, Pluronic F68 and Paclitaxel (Mw:853.91) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Chitosan (Type; Protasan UP G-113; Mw<200 kDa) was supplied from Novamatrix, Norway. All the other reagents were pharmaceutical grade.

Preparation of PCX-loaded CS/PCL NPs

PCX-loaded CS/PCL NPs were prepared according to the method used in our previous research [22]. Briefly, 10 mg PCL (Mw= 80.000) was weighed for each batch and 1 mg of PCX was added and dissolved in 5 mL organic phase (acetone). Pluronic F-68 (75 mg) and chitosan (2.5 mg) were added to ultra-pure water (10 mL) and stirred (500 rpm) at room temperature for preparing aqueous phase. At 800 rpm, organic phase was added dropwise to aqueous phase and stirring (500 rpm) continued at room temperature overnight to evaporate the organic phase completely. NPs were obtained by nanoprecipitation method. For 5 minutes, the dispersions were centrifuged at 3500 rpm. Finally, supernatants were filtered with 0.45 µm pore size filter to remove the aggregate from the solution.

Preparation and characterization of blank and PCX-loaded CS/PCL NPs dispersed in situ gel

Thermosensitive Pluronic F-127 and Pluronic F68 blank hydrogels were prepared with varying proportions of Pluronic F127. For the preparation of pluronic containing hydrogels, phosphate buffered saline was progressively mixed with the pluronic powders (PBS) (pH 7.4) under agitation on magnetic stirrer (750 rpm) at 4 °C. Formulations were mixed for 24 hours. The final pluronic concentration in the hydrogel for the formulations containing pluronic F-127 alone was 15%, 18%, 20%, 22%, and 25% w/w. For the preparation of hydrogels consisting of a mixture of F127 and F68 the ratio of F127/F68 in the formulation was determined as 19/1% [23]. After complete dissolution of the pluronic powder, each formulation

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will be equilibrated at 4°C for 24 hours to eliminate foam and air bubbles. For the preparation of nanoparticle dispersed gels PCX-loaded CS/PCL NPs were added to optimized in situ gel and mixed at 750 rpm at 4 °C. Based on the results of rheological behavior and oxygen-reactive species generation, moist heat sterilization was chosen as the method for gel sterilization [24]. After all gels were prepared, they were sterilized at 121 °C for 15 minutes in autoclave.

Gelation temperature and time

The temperature at which the sol-gel transition takes place is referred to as the gelation temperature, and the time for the start of gelation and the subsequent transition from sol to gel are also known as the gelation times (Figure 1).

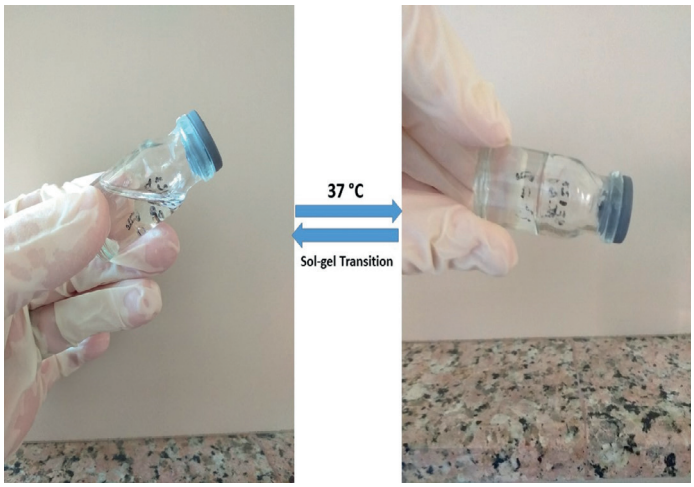

Figure 1. The sol–gel transition of a thermosensitive *in situ* gel

For gelation time, magnetic stirrer was adjusted to the range of 50-100 °C, the solutions (3 mL) were allowed to mix at 75 rpm. A thermometer was inserted into the solutions. The temperature at which the magnetic bar did not turn and the solution in the vial did not flow was determined as the gelation temperature. The gelation time was determined by taking into account the state of the gel where the solution does not flow for 1 minute after inverting a bottle. The solution (3 mL) was taken into a glass bottle and heated in a 37 ºC water bath to gel. To examine the sample's gelation after each minute, the glass vial was taken out and inverted for one minute. No flow within 1 minute after the bottle is inverted will be considered the gel state [25].

Viscosity measurements and rheological behavior of in situ gels

The viscosity measurement of the gels was determined at different rotational speeds (10-100 rpm) and constant temperature using a rotary viscometer (Brookfield DV-II) equipped with a CP52 spindle. Based on the measurements shear rate, shear stress, viscosity was determined, and flow curves were obtained.

pH and clarity

In order to determine the pH of blank in situ gel formulation and in situ gelling systems with CS/PCL NPs loaded with PCX, pH meter (Mettler Toledo, Switzerland) was utilized. Measurements for each formulation were conducted for three times (n=3). The clarity of each formulation was analyzed by visual examination under bright light with a dark background [26].

In vitro release of PCX from in situ gel formulation

In vitro release studies of in situ gelling formulations containing CS/PCX-PCL NPs were carried out by following dialysis bag method (MWCO; 14,000 Da). Dialysis membran was soaked with NaOH (%1) for overnight prior to experiment. 1000 µL in situ gel containing CS/PCX-PCL NPs was added to dialysis membrane bag and immersed into 25 mL PBS at 37°C. At predetermined time intervals, 200 µL of the release medium were taken and replaced with fresh PBS at same volume. To analyze the amount of released PCX from in situ gel, UV spectrophotometer was used at 230 nm [22].

Statistical Analysis

IBM® SPSS Statistics® V21 program was used for statistical analysis. Student's t-test was used for pairwise comparisons. In all comparisons, it was considered significant for $p<0.05$.

Results

Characterization of in situ gel formulations containing NPs

nanoparticle characterization

PCX loaded CS/PCL NPs were prepared using optimized nanoprecipitation method described in our previous study [22]. The average size of optimum nanoparticle formulation was 383.8±2.4 nm, the PDI was 0.253±0.122 and the zeta potential was +51.3±6.1. The PCX encapsulation efficiency was 77.2±2.1%.

Gelation temperature and time

Various concentrations of Pluronic F-127 were investigated in order to obtain the best concentration ratio. When the concentration of Pluronic F-127 was 15%, no gelling was observed below 37°C. When the ratio of Pluronic F-127 was increased to 18%, the gelation temperature was found to be 36.67°C ± 0.58. When the Pluronic concentration increased gelation time and temperature were decreased. Gelation temperatures and times of formulations were displayed in Table 1.

According to Kim et al., a suitable gelation temperature for in situ gel formulations should be in the range of 30-36°C [27]. F2 was selected due to its suitable gelation temperature and time. According to Aka-Any-Grah et al. F68 was added %1 to the F2 formulation and total polymer ratio kept constant at %20 [23]. The gelation time and temperature of in situ gel consisted of mixture of Pluronic F-127 and Pluronic F68 at 19%/1% ratio was found 30.33±0.58 sec and 41.67±0.58°C. The in situ gel consisted of mixture of F127 and F68 at 19%/1% was chosen as optimum formulation due to its favorable characteristics. The gelation time and temperature of optimum in situ gel containing CS/PCX-PCL NPs were found 41.33 ±0.58 sec and 30.67±1.15°C, respectively.

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Table 1. Gelation temperatures and times of formulations

Formulation	Pluronic F-127 (%)	Pluronic F68 (%)	Total pluronic ratio (%)	Gelation Temperature (°C)	Gelation Time (sec)
F1	18	-	18	36.67±0.58	57.67±0.58
F2	20	-	20	31.67±0.58	51.00±1.73
F3	22	-	22	26.33±0.58	40.00±0.00
F4	25	-	25	22.00±0.00	30.00±0.00
Optimum Formulation	19	1	20	30.33±0.58	41.67 ±0.58

Table 2. pH and clarity results of formulations

Formulation	Blank *in situ* Gel		NP Loaded *in situ* Gel	
	pH	Clarity	pH	Clarity
F1	6.09±0.05	Clear	6.15±0.02	Clear
F2	6.07±0.03	Clear	6.14±0.00	Clear
F3	6.01±0.08	Clear	6.08±0.09	Clear
F4	5.98±0.01	Clear	6.05±0.02	Clear
Optimum Formulation	6.03±0.01	Clear	6.08±0.04	Clear

Viscosity measurements and rheological behavior of in situ gels

Viscosity measurements were performed at both room and body temperature. The viscosity of blank in situ gel and in situ gel containing CS/ PCX-PCL NPs at room temperature (25 °C±0.1) were found 135.00±2.47 cP and 137.00 ±3.05 cP respectively at 100 rpm (Figure 2).

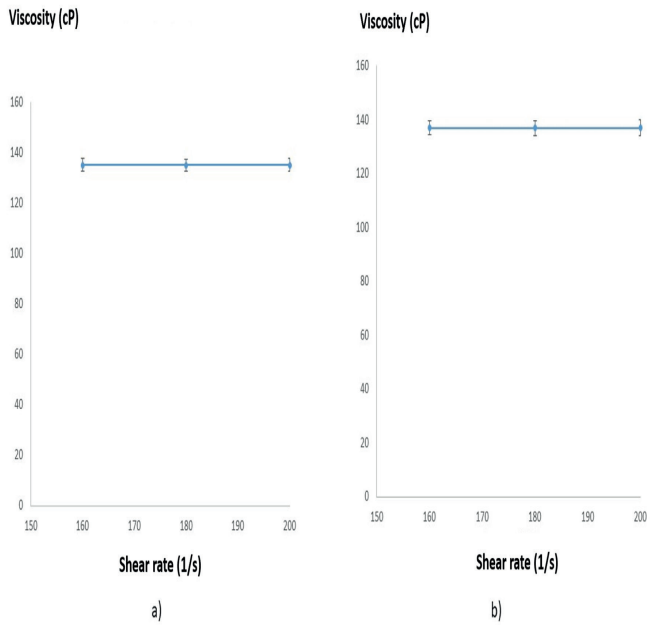

Figure 2. Flow curves of blank in situ gel a) and in situ gel containing PCX loaded CS/PCL NPs b) at 25 °C±0.1

The viscosity of blank in situ gel and in situ gel containing CS/ PCX-PCL NPs at body temperature (37 °C±0.1) were found 736.57±7.97 cP and 890.30 ±89.61 cP respectively at 100 rpm (Figure 3).

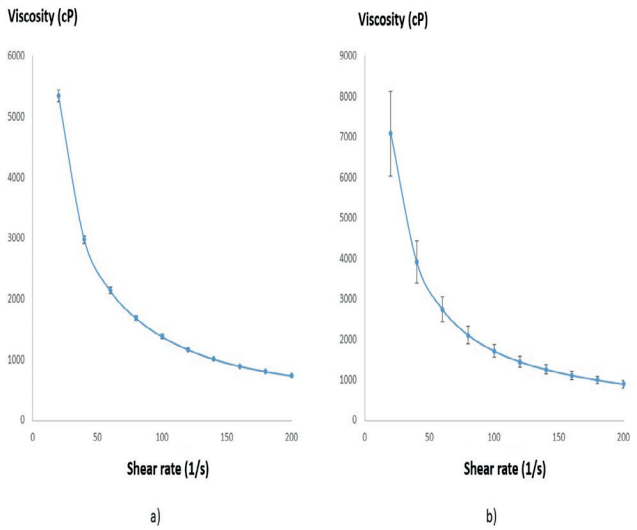

Figure 3. Flow curves of blank in situ gel a) and in situ gel containing PCX loaded CS/PCL NPs b) at 37 °C±0.1

pH and clarity

The pH and clarity results are illustrated in Table 2. The pH of our formulations varies between 5.98-6.15. According to the literature review, this pH range is accepted as suitable for injectable in situ gel formulation for bone tissue [28, 29]. Also,

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both blank and nanoparticle loaded formulations had clear appearance. Results of clarity tests indicated that nanoparticle incorporation to in situ gel formulation did not cause a significant change in the clarity of gel.

In vitro release studies

Figure 4 demonstrates the cumulative release of PCX from PCL NPs and in situ gel containing PCL NPs. According to the in vitro release study, PCL NPs formulation released PCX faster than in situ gel containing PCL NPs. For the PCL NPs alone, higher than %70 of PCX was liberated from nanoparticle system at the end of the release experiment. İn situ gel formulation, on the other hand, still maintained approximately %40 of PCX at 96 h. It can be clearly observed that in situ gel formulation could extend the release of PCX compared to PCL NPs alone.

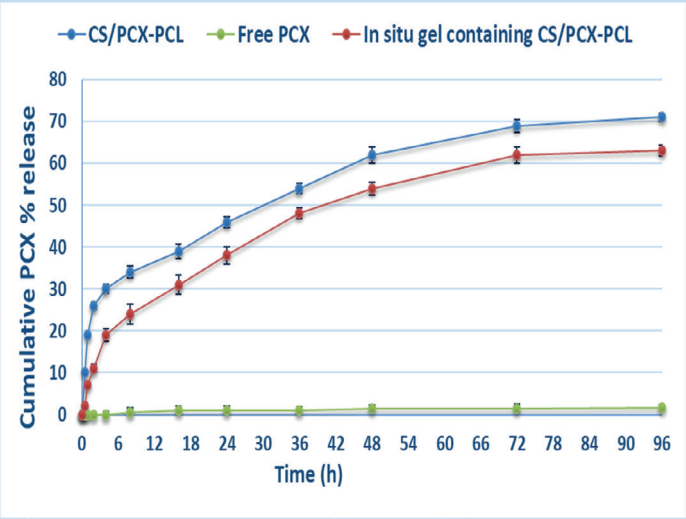

Figure 4. Cumulative release of PCX from CS/PCX-PCL and in situ gel formulations containing CS/PCX-PCL

Discussion

Local therapeutic approaches have recently aroused a great deal of attention. Since it allows to directly reach to target area, has led to low adverse effects and increases treatment efficiency, localized treatment approaches have been commonly preferred lately. İn situ gelling systems are among the most effective candidates which are able to provide localized therapy. They offer a wealth of benefits for treatment including extended-release profile, convenient administration, increased retention time on the tissue. In this context, in situ gel formulations containing CS/PCX-PCL NPs were developed to provide an effective way to be used in cancer type metastasis to bone tissue.

CS/PCX-PCL NPs were developed by following the method developed in our previous study [22]. The NPs had ideal properties in terms of size, PDI, ZP and encapsulation efficiency. According to gelation temperature and gelation time results, increased Pluronic F-127 concentration used in formulations has led to lower gelation time and temperature. Physiological temperatures will cause the formulation to have liquid qualities if the gelation temperature is too high, which can cause leakage. Lower gelation temperatures, on the other hand, may cause application chalenges due to the viscous nature of the formulation. In the light of these information, F2 was selected as the optimum formulation. Then, to obtain lower gelation time, Pluronic F-127 and Pluronic F 68 were mixed at 19%/1%. The addition of the active substance or additives is known to change the sol-gel transition temperature [30]. Furthermore, short gelation time is important in terms of preventing drainage from the application site of the active substance and the residence time in the application area for prolonged release. However, the addition of active substance had no effect on gelation temperatures and gelation times in our in situ gels. No significant differences were found in gelation time and temperature of between blank in situ gel and in situ gel containing CS/PCX-PCL NPs ($p>0.05$).

İn situ gel formulations should ideally have a low viscosity in sol state so that the formulation can be easily injected at the application site. On the other hand, after application, the in situ gel formulation must have a high viscosity at body temperature so that it comes into contact with the bone tissue and stays longer in the defect area to ensure effective bone regeneration [31]. Based on our viscosity results, blank in situ gel and in situ gel containing CS/PCX-PCL NPs are suitable. Flow curves were shown Figure 2 and Figure 3. The results indicated that, poloxamer solution showed a Newtonian behavior at room temperature while it might be a pseudoplastic fluid when the temperature reached above gelation temperature at body temperature. Poloxamer molecules exist as monomolecular micelles in an aqueous solution at low temperatures, surrounded by a sheath of hydrogen bonded water molecules, with hydrophilic poly(oxyethylene) moieties wrapped around central hydrophobic poly(oxypropylene) moieties. There are few interactions between polymer molecules in this case the chains are highly mobile. As a result, poloxamer solutions have Newtonian flow properties in whichviscosity keeps constant when shear rate increases. Our results are compatible with literature [32]. As the temperature rises, the hydrophobic poly(oxypropylene) sections of the poloxamer chains' hydrogen bonds become unstable and eventually desolvate, allowing for widespread hydrogen bonding between poly(oxypropylene) moieties on nearby chains. The hydrophobic core of multimolecular micelles is formed by poly(oxypropylene) aggregates, with the hydrophilic poly(oxyethylene) portions interacting with the aqueous vehicle. The formation of multimolecular micelles causes a reduction in chain mobility [33] As a result, above gelation temperature, poloxamer gels exhibit pseudoplastic flow characteristics. In pseudoplastic flow characteristics, when the shear rate increased, the viscosity decreases. Our results are compatible with literature [34-36]. Furthermore, our pH and clarity results pointed out that our formulations are in acceptable range and addition of CS/PCX-PCL NPs did not result in significant changes ($p>0.05$).

When release data were examined, it is obvious that in situ gel notably increased the release time of PCX compared to CS/PCL NPs. The reason why in situ gel loaded with CS/PCL NPs exhibited

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prolonged release profile can be explained by advantages provided by in situ gel systems. Process of drug release from in situ gels containing NPs carries out in two steps. At first, in situ gel disrupts gradually and collapse totally. During that time, NPs carried by in situ gel formulation liberates from gel matrix. In the second step, drug molecules release from NP system. Accordingly, in situ gel released the drug slower than CS/PCL NPs alone. When graphics were examined thoroughly, 2 stages of release are noticed; fast release in the initial of experiment and prolonged release over an extended period. In the first 6 hours, the burst effect was observed. The main reason of burst effect is because of surface modification with CS. Since CS covers the surface of the nanoparticle, drug molecules are adsorbed by CS. Therefore, after the first interaction between nanoparticles and release medium, firstly drug molecules adsorbed onto CS release from nanoparticle system and as a result, faster drug release occurs. Our results are compatible with the literature [37].

Conclusion

In the present study, the PCX with poor solubility was loaded to PCL NPs and then NPs were incorporated to in situ gel formulation in order to provide prolonged release profile, increased retention time in tissue. In this context, characterization tests of PCL NPs and in situ gel formulation were conducted. PCL NPs had optimum properties with the particle size 383.8±2.4 nm, the PDI 0.253±0.122 and the zeta potential +51.3±6.1 mV. To obtain a gel formulation with ideal gelation time and temperature various pluronic ratios were evaluated. It was seen that as concentration ratio of Pluronic F-127 was increased, gelation time and temperature were decreased. Based on the results, optimum formulation was selected as F2 containing %20 Pluronic F-127. Subsequently, in order to decrease gelation time, Pluronic F 68 was added at the rate of %1 by keeping total pluronic (Pluronic F-127 + Pluronic F 68) ratio at %20. pH and clarity results were suitable for injection to targeted tissue. According to release data, it was proven that in situ gel formulation could achieve sustained PCX release. This study has been a promising preformulation study for injectable in situ gel formulation to be utilized in bone metastasis.

Conflict of interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
The authors declare that they have received no financial support for the study.

Ethical approval
It does not require ethics committee approval. No ethical rules are required in terms of the scope of the study.

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