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In Situ Gels Triggered by Temperature for Ocular Delivery of Dexamethasone and Dexamethasone/ SBE-*β*-CD Complex

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ABSTRACT: This research aims to increase the solubility of Dexamethasone (Dex) using cyclodextrin and develop a temperature-triggered in situ gelling system for the ocular application. The solubility of Dex was increased with SBE-β-CD. Dex- SBE-β-CD inclusion complex was prepared with kneading and freeze-drying method. Structural characterization was carried out using DSC and FT-IR. When in situ gel formulations were prepared, Pluronic F127 (PF127), a thermosensitive polymer, and Chitosan (CH), a natural, biodegradable, and mucoadhesive hydrophilic polymer, were used together. When the solubility diagrams of the drug-cyclodextrin inclusion complex were examined, it was determined that SBE-β CD showed a linear increase, and AL-type diagram was selected in consequence. The formulations were produced using different amounts of PF127 and a fixed ratio of CH. In situ gels were evaluated for clarity, pH, gelation temperature, and rheological behaviors and selected one formulation. It was established that the formulations were clear, their pH was 6, their gelation temperature decreased with increasing PF127, and was between 22-34 °C. For the selected formulation, 0.1% Dex and Dex/ SBE-β-CD were transferred to in situ gelling systems. As a result of in vitro release studies, it was observed that the release of the Dex/SBE-β-CD inclusion complex containing BRN formulation showed a higher burst effect than the other and was released for 6 hours. The results exhibited that the combination of PF127 and CH has potential as an *in situ* gelling systems for ocular delivery of Dex and Dex/ SBE-β-CD.

KEYWORDS: Dexamethasone, *In Situ* Gel, Ocular Drug Delivery, SBE-*β*- Cyclodextrin, Chitosan

1. INTRODUCTION

The eye has a unique anatomy, so it is one of the most challenging organs for drug delivery. The barriers of the eye make it challenging to deliver drugs to the deeper tissues. Conventional eye drops contain 90% of the ocular drugs on the market, and these products are administrated into the eye with the linearity of doses. Although, these eye drops have disadvantages, such as rapid corneal elimination by the ocular barriers and limited residence time [1].

In recent years, drug delivery systems called "in situ gel" have been developed and widely used in ophthalmic formulations. Specific polymers that undergo sol-gel phase transition by induction of ambient conditions such as pH [2], specific ions [3], and temperature [4] are used in the preparation of *in situ* gels. *In situ* gel formulations are initially prepared as solutions or suspensions and turned to gel where they are administrated to increase patients' compliance [5]. Studies have demonstrated that corneal residence times of some *in situ* gel systems can be up to a few hours. Different polymer or polymeric combinations have been used successfully to adjust the release profile as desired [5].

One of the polymers used *in situ* gel systems is pluronics (poloxamer) and has a thermoresponsive structure. It exhibits amphiphilic behavior because of the hydrophilic ethylene oxide area and hydrophobic propylene oxide area. The gelation of poloxamers could be described by the observed differences in micellar structure depending on temperature and concentration. Poloxamers have been extensively used as an ophthalmic drug delivery system since they could prolong drug release and possess sufficient inert character for the eye. On the other hand, a primary obstacle of poloxamer is insufficient mucoadhesive property; therefore, additional polymers have been used to increase mucoadhesive characters of the poloxamer-based

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ocular formulations, such as sodium hyaluronate, carbopol, and chitosan (CH) [6]. CH demonstrates suitable properties for ocular application since it is a cationic, biocompatible, biodegradable polysaccharide. Also, it has been exhibited to have good mucoadhesive properties and antibacterial activity [7].

Dexamethasone (Dex) is a steroidal agent widely utilized to treat inflammatory conditions in the clinic and is a lipophilic drug with low solubility (100 µg/mL) and high permeability [8,9]. Based on the Biopharmaceutical Classification System (BCS), such drugs are classified as class II compounds [10]. Furthermore, Dex eye drops are quickly removed from the eye's surface in a short time (approximately five minutes) after topical application due to precorneal protective factors and relative impermeability of the corneal epithelial layer, which cause poor bioavailability [11]. Therefore, marketed Dex eye drops require a more frequent application to maintain effective therapeutic concentration.

One of the approaches used to increase the solubility of lipophilic drugs such as Dex is preparing drug-cyclodextrin complexes. Cyclodextrins (CDs) are generally preferred to increase the water solubility of drugs with low water solubility and increases corneal permeability It has been determined that Dex-cyclodextrin inclusion complexes are formed by using different cyclodextrin (CD) derivatives. Nevertheless, Dex/sulfobutyl ether-β-cyclodextrin (SBE β-CD) and Dex/β-cyclodextrin inclusion have not been reported yet [12]. When the literature was examined, it was determined that SBE-β-CD had higher complexation efficiency and higher solubility capacity than other β-CD derivatives [13]. CD are preferred when developing ocular drug delivery systems to increase corneal permeability along with drug solubility to enhance ocular bioavailability [14]. It exhibits favorable therapeutic effects and rarer ocular irritation. Moreover, it was observed that the CD-formed complex with diethyldithiocarbamate and disulfiram reduced the side effects (cataract formation) compared to the free drug in a study [15]. Therefore, it is suitable for ocular formulations of low soluble drugs.

The main aim of this study is to increase the solubility of Dex with CD. Two different CD derivatives, SBE β-CD and β-CD, were used for this objective. Based on the phase-solubility study results, a suitable CD was selected to increase Dex's solubility. *In situ* gels containing different ratios of PF 127 (15%-20%-25%) and constant CH were prepared. In addition, developed gel formulations were evaluated for clarity, pH, gelation temperature, and rheological behaviors, and a suited formulation was selected. For the selected formulation, 0.1% Dex and 0.1% SBE-β-CD–Dex inclusion complex were added to *in situ* gelling systems, and release of Dex was evaluated to the usage of designed *in situ* gelling systems for ocular delivery.

2. RESULTS AND DISCUSSION

2.1. Stability of cyclodextrin-drug complex

Phase-solubility studies are usually the preferred method for determining the efficacy of drug-CD complexation on drug solubility [16]. The 1:1 drug/CD inclusion complex is the most frequent type of association when the drug molecule is incorporated into the cavity of a CD molecule, with a stability constant K1:1 for the equilibrium between free and associated species. When the solubility diagrams were examined, it was determined that SBE-β-CD and β-CD had a linear increase (Figure 1). According to the phase-solubility diagram, it was decided to classify the SBE-β-CD and β-CD diagram as "AL-type". By examining the straight line of SBE-β-CD (r^2=0.9784) and β-CD (r^2=0.8689) (Fig. 1), the slope was determined as 0.2971 and 0.17, respectively. Complexation efficiency (CE) and stability constant (Ks) of SBE-β C were calculated as 0.42 and 1660 M^{-1}, and CE and Ks of β-C were 812 and 0.2, respectively. Based on the literature, Ks' value between 100 and 10,000 M^{-1} is ideal for forming the drug:CD complex. The two cyclodextrin derivatives also have Ks in this range, however, SBE-β CD increased solubility 11-fold while β-CD 7-fold. Therefore, it was decided to continue the study with SBE-β CD. Besides, the drug:CD complex ratio was decided to be 1 mM:1 mM when the AL type solubility curve was checked [17].

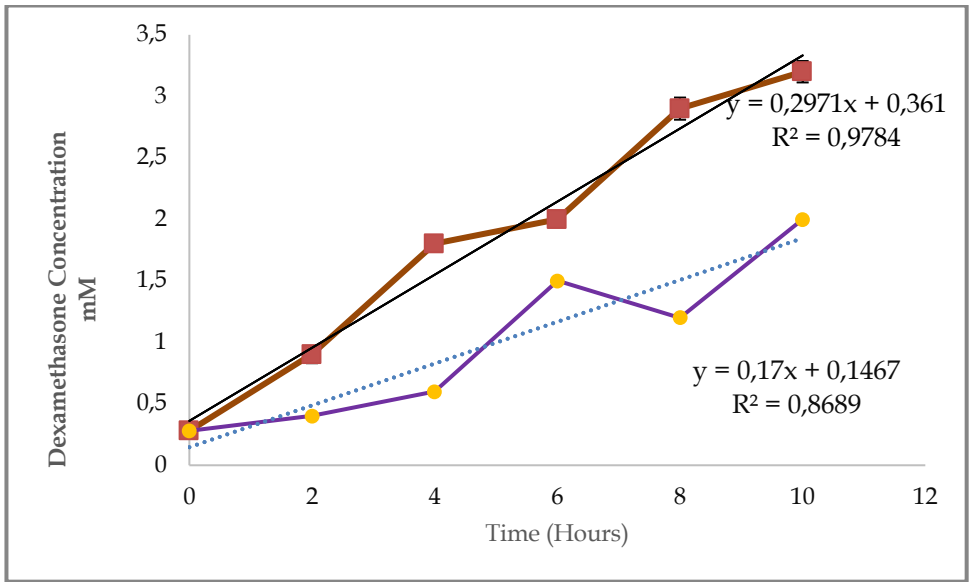

Figure 1: Phase-solubility diagram of dexamethasone at the increasing SBE-*β*-CD and *β*-CD concentration

DSC results of Dex, SBE-β-CD, physical mixture (Dex-Phy), inclusion complexes prepared by kneading method (Dex-Knd), and by freeze-drying method (Dex-Fzd) were shown in Figure 2. The thermogram of Dex exhibited a typical endothermic peak with an onset of melting at 229.5°C. When the Dex Phy, Dex-Knd was examined, it was seen that the specific peak of Dex did not disappear while it disappeared in the Dex-Fzd [18].

When the Dex FT-IR result was evaluated, the spectrum consisted of the broad absorption band around 2900–3400 cm^{-1} related to the stretching of aliphatic C-H bonds as well as the absorption band at 1650 cm^{-1} designated to C=O stretching vibration [19]. When Dex-Fzy was checked, it was seen that the specific peaks of Dex vanished contrary to Dex-Phy and Dex-Knd (Fig 3). Also, the peaks of SBE-β-CD were preserved, demonstrating the successful formation of the Dex-SBE-β-CD complex.

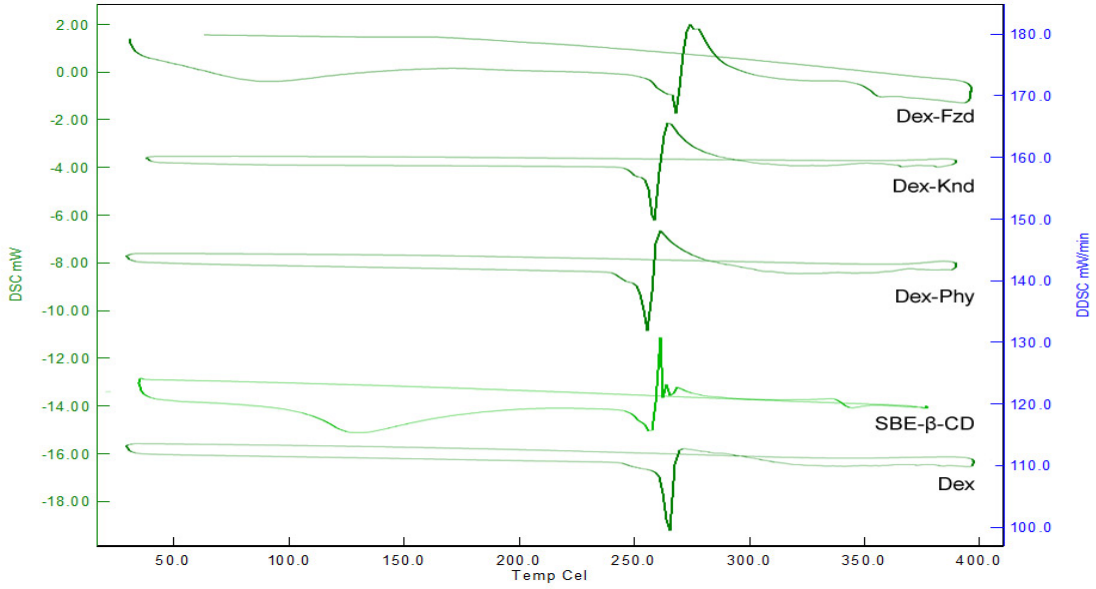

Figure 2: DSC thermogram of Dex, SBE-*β*-CD, Dex-Phy, Dex-Knd and Dex-Fzd

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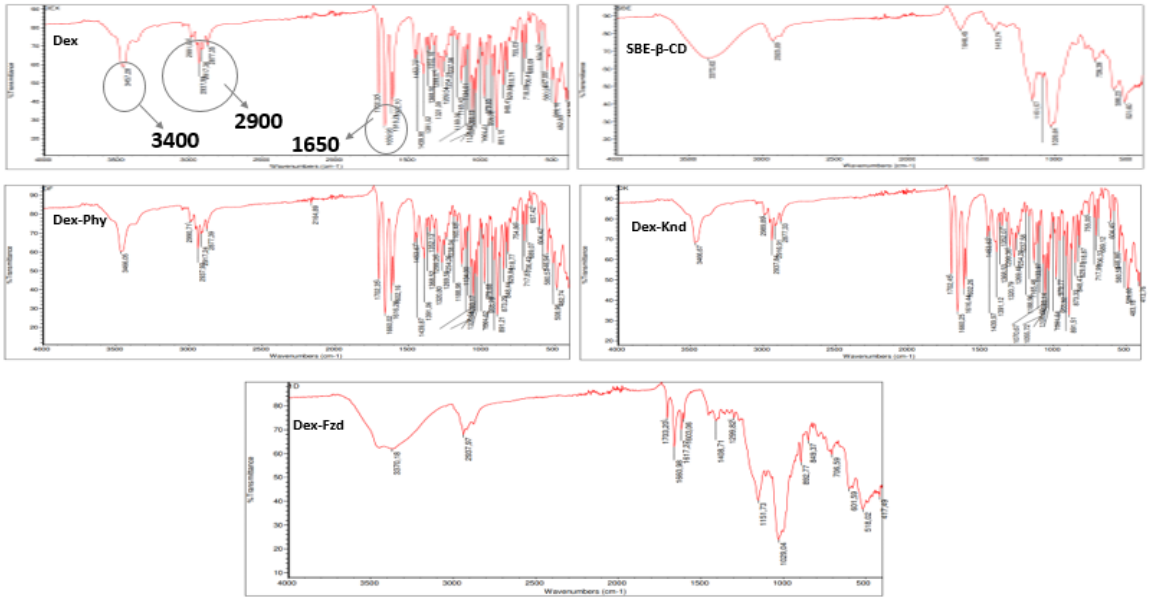

Figure Figure 3: FTIR spectra of Dex, SBE-*β*-CD, Dex-Phy, Dex-Knd and Dex-fzd

2.2. Gelation Temperature and Clarity

In situ gels must have a low viscosity between 4 °C and 25 °C; thus, they can easily apply like eye drops. However, *in situ* phase transition is required to establish a transparent gel between 30-37°C. Clarity is a critical quality of *in situ* gel as it enhances patient acceptability [20]. In this study, aqueous solutions that included PF127 at different ratios were produced to determine the appropriate formulation for *in situ* gel systems. When the *in situ* gel formulations were examined, it was determined that all of them were clear.

It was seen that the gelation temperature of T1 and T2 were lower in developed gel formulations and were observed as 22°C and 25°C, respectively. It was noticed that the gelation temperature raised due to the decline of the PF 127 concentration by 15%. The gelation temperature of the T3 formulation is 34 °C.

In an aqueous environment, Pluronics keeps each molecule separate at temperatures under the critical micelle temperature (CMT) at which the critical micelle concentration (CMC) takes place. When the temperature rises above CMT, the molecules are triggered to form micelles surrounding the hydrophobic core with hydrophilic pluronic chains in contact with the aqueous medium. As a result, increased Pluronic concentration leads to a lower CMT value [20].

This situation also has severe effects on the gelation temperature. The gelation temperature is mainly correlated to the polymer concentration of poloxamer. They form monomolecular micelles at lower concentrations while multimolecular lattice structures at higher concentrations [21]. In our formulations, concentrations of pluronic have been reduced to provide gelation is at corneal temperature. Therefore, gelation temperatures increased with the decrease of pluronic concentration in T1-T3 formulations. Similar results were observed in the literature [22].

2.3. pH Value

pH is one of the significant parameters for all ocular formulations. The pH value must be between 5.0 and 7.4 so that ocular drugs should not cause eye irritation [23-24]. When all formulations' pH was measured, they were observed at approximately 6.0.

The formulations' pH was around 6.0 because the CH solutions were prepared by dispersing them in acetic acid (1% w/v). Usage of the acetic acid solution in developed formulations had decreased the pH.

2.4. Viscosity

Viscosity is essential for in vivo effectiveness and usage for *in situ* gels. Elevated viscosity values may cause issues in the application, while low values may cause rapid removal of formulations from the eye surface. Formulation with pseudoplastic behavior is usually favorable to overcome high ocular, interblinking, and blinking shear rates. The pseudoplastic flow of liquids assures comfortable application, at the same time, manages discharge as a result of extended corneal contact time [25].

Results demonstrated that the changes in angular velocity affected the viscosity (Fig 4). According to the rheological behaviors, all formulations at their gelling temperatures were exhibited pseudoplastic flow (shear thinning system) similar to tear fluid.

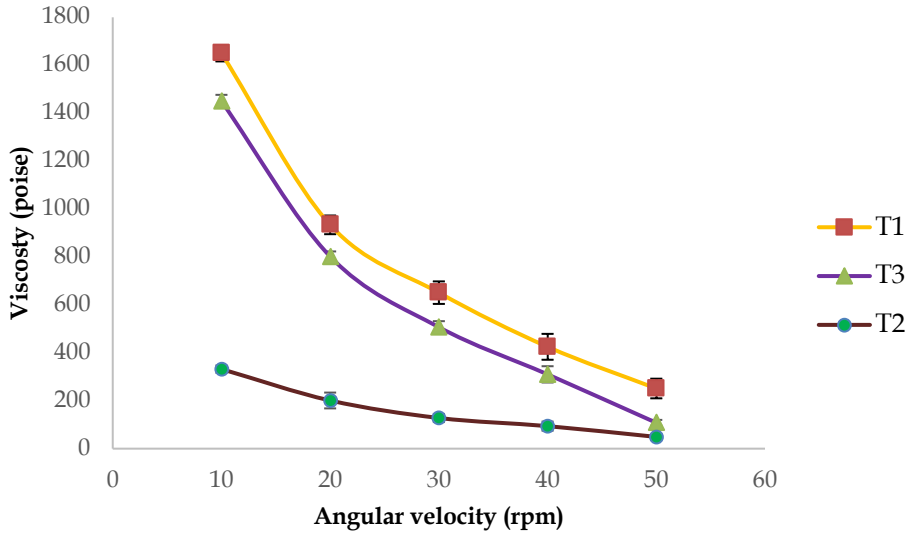

Figure 4: Rhelogical profiles of in situ gelling systems.

Ocular solutions' optimum viscosity range changes 50–50,000 mPa.s after the gel construction when the literature is checked [26]. Nevertheless, viscosity values at 10 rpm were also operated for comparative evaluation. It was concluded that the PF127 concentration had a substantial effect on the viscosity of the gels. When the PF127 concentration increased from 15% to 25%, the viscosity of the gels increased from 332 to 1650 poise at 25 °C, revealing that PF 127 enormously influences the viscosity at raising concentrations.

Among all formulations, the optimized formulation was selected according to pH value, gelation temperature, clarity, and viscosity. Dex (0.1 % w/v) Dex/SBE-β-CD cyclodextrin inclusion complex (0.1 % w/v) was added to *in situ* gelling formulations, chosen to be suitable. These are shown in Table 1 as NK, BRN. The formulations' appearance was clear, pH values were between 5.9-6.0, their gelling temperatures were 34 °C, and their viscosity was raised by increasing temperature. While the viscosity value of *in situ* gels was around 300 Poise at 25 °C, it raised to approximately 1600 Poise at 35 °C.

All *in situ* gel formulations' rheological behaviors showed pseudoplastic flow at gelling temperatures. In addition, it was encountered that similar results were obtained in comparable studies [27]. Besides, the high viscosity of an ocular formulation is undesirable as it tends to leave a distinct residue on the eyelid side after application.

Table 1: Physical properties of drug containing formulations and their component.

Formulation components and Physical Properties	NL	BRN
Dexamethasone (% w/v)	0,1	-
Dexamethasone-SBE-*β*-CD Cyclodextrin (% w/v)	-	0,1
PF 127	15	15
CH (%1 w/v)	10	10
pH (±SD)	6,0±0.01	5,9±0.02
Gelation temperature (° C±SD)	35±0.4	35±0,3
Viscosity (poise) 25 °C	330±15,5	320±18,8
Viscosity (poise) 35 °C	1624±32,3	1637±40,8
Clarity	Clear	Clear

2.5. Drug Release

All formulations were Dex loaded (0.1% w/v), and *in vitro* drug release of *in situ* gel was conducted in isotonic phosphate buffer pH 7.4 at 35°C. In vitro drug release profiles were demonstrated in Figure 5. NL exhibited > 55% drug release after two hours, and at the end of 3 hours, 70% of the Dex was released. BRN exhibited > 70% drug release after two hours due to cyclodextrin in the formulation.

In vitro release studies showed that the BRN formulation was released quicker than the NL formulation due to the Dex-SBE-β-CD inclusion complex in the BRN formulation. The formation of drug-CD increased the drug's solubility, which, notably, affected the release time here. When the literature was examined, it encountered similar results. For example, a study by Polat et al. produced insert formulations containing Besifloxacin HCl and Besifloxacin HCl-CD inclusion complex. As a result of the release studies, it was determined that the release rate of the insert formulation containing the drug-CD complex was higher than containing the bare drug [20].

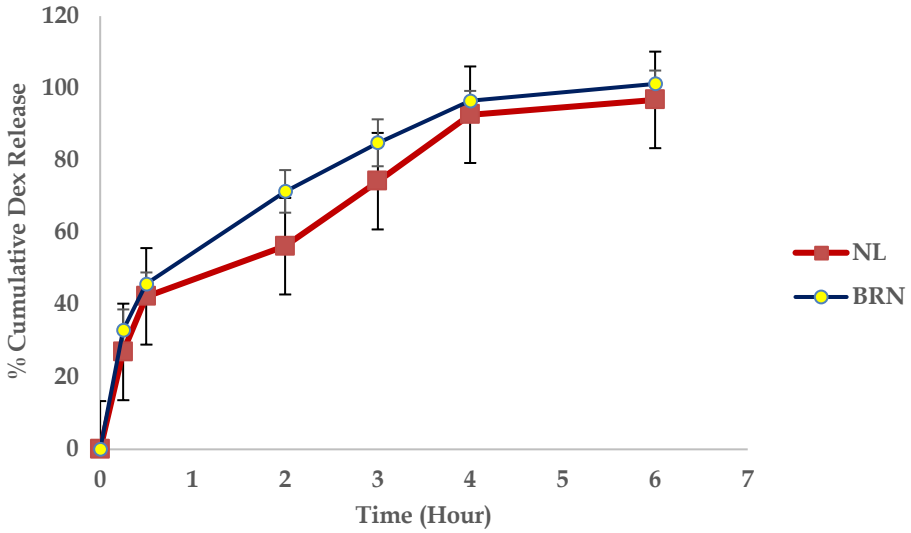

Figure 5: *In vitro* release profiles of Dex (NL) and Dex/ SBE-*β*-CD (BRN) loaded *in situ* gel formulation

The analysis and modeling of drug releases become complicated with polymer in formulation involved [28]. In situ gel formulations with swelling polymers such as poloxamer and chitosan show diffusion or erosion-controlled mechanism. Often the two mechanisms are observed together [28].The drug release kinetics of Dex from in situ gel was calculated by using non-linear regression model of KinetDS. The parameters and determination coefficients (R2) calculated with this method are shown in Table 2. In the study, evaluation was made according to different models such as zero-order, first-order, Higuchi, Hixson–Crowell, Korsmeyer–Peppas and Weibull models. As can be seen from the regression coefficients, the release kinetics for NL and BRN formulation are best described by the Korsmeyer–Peppas model. In literature, there are

several studies that experimentally investigated and indicated that the release data from swellable polymeric nanoparticles fits best with the Korsmeyer–Peppas model [29-30].

Table 2: In-vitro release kinetic parameters of Dex from In situ Gel

Sample	Zero Order (R^2)	First Order (R^2)	Higuchi (R^2)	Korsmeyer-Peppas (R^2)	Weibull (R^2)	Hickson-Crowell (R^2)
NL	0.9085	0.8322	0,8589	0,9616	0,7132	0,8643
BRN	0,8478	0,7798	0,664	0,9836	0,7579	0,8051

3. CONCLUSION

In this study, it was used different cyclodextrins derivatives. It was found that SBE-β-CD and β-CD increased the solubility of Dex by 11 and 7 times, respectively, so SBE-β-CD was chosen. Inclusion complexes of drug-SBE-β-CD were produced with different methods. As a result of DSC and FT-IR studies, it was determined that the production was successfully carried out via the freeze-drying method. At the same time, various *in situ* gelling systems containing different ratios of PF127 and a constant amount of CH were produced. All formulations were characterized in terms of pH, clarity, gelation temperatures, and rheological behavior. Their rheological behavior showed that all formulations had pseudoplastic flow similar to tears. The gelation temperature and viscosity of the T3 formulation were determined as 34±0.9 °C and 332±15.4 poise, respectively, and concluded that it was suitable for the ocular application. Subsequently, the optimized formulation was loaded with Dex and Dex-SBE-β-CD. In this formulation, the pH was 6.0, the gelation temperature was 34 °C, and it demonstrated pseudoplastic behavior. Moreover, it exhibited up to 6 hours of drug release. Dex is a steroidal agent with high potency and effectiveness widely utilized in treating inflammatory conditions; however, it might cause severe side effects because patients do not use it as desired. *In situ* gel provides prolonged residence time of the drug on the eye, and the drug would be released longer than conventional eye drops.

4. MATERIALS AND METHODS

4.1 Materials

Dexamethasone, pluronic F-127 (PF 127), chitosan (low molecular weight), SBE-β-CD, β-CD, phosphate-bufferedsaline (PBS) tablet, acetonitrile (ACN) and methanol (MeOH) were purchased from Sigma, Steinheim, Germany.

4.2. Production of cyclodextrin-drug complex

4.2.1. Cyclodextrin-drug phase-solubility studies

The phase-solubility was based on that developed by Loftson et al. [16]. Increasing concentrations (0-10 mM) of SBE-β-CD solution or β-CD solution were added into a fixed amount of Dex. The resulting mixture was stirred at room temperature for seven days with a magnetic stirrer. At the end of the seventh day, all mixtures containing SBE-β-CD or β-CD and Dex were filtered using 0.45 µm membrane filters. The amount of Dex in the supernatant was concluded by HPLC method. The HPLC (Thermo Scientific, USA) was carried out C 18 column (75 mm × 4.6 mm, 5 µm) with a mobile phase of ACN:MeOH:Water (25:25:50 v/v/v%) at a flow ratio of 1 mL/min. The wavelength used to detect Dex in the mixture was 254 nm. The injection volume for all samples was 10 µL. The column temperature was kept fixed at 25 °C throughout the analysis. The experiment was performed in triplicate (n = 3). At the end of the study, the phase-solubility diagram was depicted by presenting SBE-β-CD or β-CD concentration against the dissolved Dex amount [31].

In addition, Phase-solubility diagrams can be classified in different ways [32]. This classification contains AP, AL, AN, BS, and BI diagram models. Complexation efficacy and complex stability constant were determined by Loftson et al. calculated according to the equation used [16].

The complex stability constant was figured according to Eq. (1) [16]

$$\text{Complex stability constant} = \frac{Slope}{S0(1-slope)}$$

intrinsic Dex solubility is shown as S, which is 0.25 mM, and the linear regression's slope of the phase-solubility diagram is shown as Slope.
The complexation efficacy was calculated according to Eq. (2) [16]

Complexation efficacy = $\frac{Slope}{(1-Slope)}$

4.2.2. Production of cyclodextrin-drug complex.

Cyclodextrin-drug inclusion complexes were produced with two different methods in order to evaluate the effect of different methods on complexation. One of these methods is kneading, and the other is the freeze-drying method.

Kneading

Equimolar amounts of Dex and SBE-β-CD were used. The CD was wetted in a mortar, and Dex mixed. Then, the required amount of ethanol/water (3:1 v/v) mixture was added to maintain the appropriate consistency of the mixture and kneaded in a mortar for 45 minutes. The final product was dried in an oven (hot air oven, Nuve, FN 055/120, Holland) at 25± 0.5°C for 24 hours [33].

Freeze-drying

The freeze-drying method was used to produce cyclodextrin-drug complexes [34]. Each of the SBE-β-CD and Dex substances (equal molar ratio of 1:1) was dissolved in either water or ethanol, respectively. It took 24 hours for the SBE-β-CD and Dex solutions to be mixed before ethanol was evaporated, then later sample was lyophilized. FT-IR and DSC were used to check the development of inclusion complexes are succeeded.

4.3. *Production of in situ gel*

A modified cold method was used to prepare the *in situ* gel formulations [35]. All PF127 solutions (15, 20%, 25% w/v) used in this study were prepared by mixing the polymer with cold (4°C) water. The polymer solutions were kept for 24 hours in the fridge. Then, the CH (1% w/v) solution was prepared for formulations. First, CH was dissolved in acetic acid solution (2% v/v), then the CH solution was kept for 24 hours in the fridge. Next, the CH solution was added to the PF127 solution at the same temperature. Each example was stored at 4 °C before use. The produced in situ gel formulations are demonstrated in Table 3.

Table 3: Components of *in situ* gelling formulation

İn situ gel components	T1	T2	T3
PF 127 (%w/v)	25	20	15
CH (1% w/v acetic acid) ml	10	10	10
Water q.s. to ml	100	100	100

4.4. Characterization of *in situ* gel formulations

Solutions of different concentrations of PF 127 together with CH (formulation codes T1–T3) were determined for gelation temperature, pH, clarity, and viscosity. Obtained results are shown in Table 4.

4.5. pH

pH measurements were carried out by a pH meter (HANNA, Germany). The measurements were performed in triplicate (n=3).

4.6. Clarity

The clarity of the *in situ* gels formulation after gelation was determined by examining them under intense light on a black background [36] (Table 4).

Table 4: Physical properties evaluation results of *in situ* gelling systems

Formulation	pH (±SD)	Gelation Temperature (°C±SD)	Viscosity (poise) 25 °C	Clarity
T1	5,9±0.02	22±0.8	1650±34,4	Clear
T2	5.9±0.01	25±1.4	1450±26,5	Clear
T3	6,0±0.02	34±0.9	332±15,4	Clear

4.7. Gelation Temperature

All polymer solutions (10 ml) were stirred in a water bath with a magnetic stirrer. The polymer solutions were heated at 1 °C/min, stirring at 100 rpm (Thermomac-TM19). Each measurement was performed three times.

4.8. Viscosity

The viscosity of *in situ* gels was measured by using Brookfield, DV2T-RV Viscometer (Essex, UK) with CP 52 spindle. The spindle runs at 1, 2.5, 5, 10, 20, 50 rpm angular velocity. Viscosities of in situ gels were measured at their gelation temperature. Viscosities were measured at different angular velocities, and flow curves were determined. Viscosity at 10 rpm was also shown for comparative evaluation (Table 4). The experiment was performed in triplicate.

4.9. Production of Dex Contained *In Situ* Gel Formulation

All formulations were evaluated for their physical properties, and suitable formulation has been determined. Dex and Dex-SBE-β-CD inclusion complex were added to chosen suitably in situ gel formulation. These two formulations were named NL and BRN. Dex is available as a 0.1% (w/v) ophthalmic suspension in commercial formulations; thus, it was used in situ gel formulations at a drug concentration of 0.1% Dex, and Dex-SBE-β-CD inclusion was mixed with selected in situ gel formulation. Recorded formulations, including Dex and their physical properties, are demonstrated in Table 1.

4.10. *In Vitro* Release Studies

The dialysis bag approach was used to undertake in vitro release tests on in situ gel formulations [37]. Briefly, the dex-loaded formulation (100 µL) was added to the dialysis bags, and they were closed and placed in 25 mL of pH 7.4 isotonic phosphate buffer at 37 °C. In this way, the sink condition is provided. Equal aliquots of medium were taken at different time intervals (15, 30, 60, 120, 180, 240, 360 min), and equal aliquots of the new buffer medium were transferred to replace the withdrawn examples. Dex concentrations were determined by HPLC. Dex release profile was depicted according to the total quantity of drug released from each formulation over time. The experiment was performed in triplicate.

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Author contributions: Concept – K.P; Design – K.P.; Supervision – K.P.; Resources – K.P.; Materials – K.P.; Data Collection and/or Processing – K.P.; Analysis and/or Interpretation – K.P.; Literature Search – K.P.; Writing – K.P.; Critical Reviews – K.P.

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