



# Thermosensitive *In situ* Gelling System for Dermal Drug Delivery of Rutin

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## ABSTRACT

**Objectives:** Rutin has been broadly applied for treating several diseases due to its pharmacological activities. However, its low aqueous solubility limits its absorption and bioavailability. This research aims to increase the solubility of rutin using cyclodextrin and to develop a temperature-triggered *in situ* gelling system for dermal application.

**Materials and Methods:** The solubility of rutin was increased with sulfobutyl ether- $\beta$ -cyclodextrin (SBE- $\beta$ -CD). Rutin-SBE- $\beta$ -CD inclusion complex was prepared by kneading and freeze drying method. Structural characterization was carried out using differential scanning calorimetry and fourier transform infrared spectroscopy. *In situ* gel formulations were prepared with pluronic F127 (PF127), a thermosensitive polymer, and chitosan (CH), a natural, biodegradable, and mucoadhesive hydrophilic polymer. *In situ* gel characteristics such as pH, clarity, gelation temperature, and viscosity were determined.

**Results:** When the solubility diagrams were examined, it was concluded that SBE- $\beta$ -CD showed a linear increase, therefore, AL-type diagram was selected. The formulations were produced using different amounts of PF127 and a fixed ratio of CH. Three *in situ* gels were evaluated for their pH, gelling temperature, and the rheological behaviors, and one formulation was selected. It was observed that the formulations had a pH between 6-6.1, and their gelation temperature decreased with increasing PF127 that was between 20°C to 34°C. For the selected formulation (formulation E3), 0.5% rutin and rutin/SBE- $\beta$ -CD were transferred to the *in situ* gelling system. Because of *in vitro* release studies, it was observed that the release of the rutin/SBE- $\beta$ -CD inclusion complex containing NZ formulation showed a higher burst effect than the others and the release continued for 6 hours.

**Conclusion:** The results indicated that the combination of PF127 and CH can be a hopeful *in situ* gelling vehicle for dermal delivery of rutin and rutin/SBE- $\beta$ -CD.

**Key words:** Rutin, *in situ* gel, dermal drug delivery systems, SBE- $\beta$ -cyclodextrin, pluronic

## INTRODUCTION

Flavonoids are secondary metabolites with phenolic structures found in many plants. According to their molecular structures, flavonoids are divided into varieties such as flavones, flavonols, isoflavones, neoflavonoids, flavans, flavanones, flavanonols, anthocyanidins, aurones, and chalcones. Flavonoids have many pharmacological activities, such as anticancer, anti-inflammatory, antioxidant, hypoglycemic, diuretic, and hepatoprotective.<sup>1</sup>

Rutin (quercetin-3-O-rutinoside) is in the form of flavonol glycoside, and is also known as vitamin P, and has a structure of 5,7,3',4'-tetrahydroxyflavone-3-rhamnoglucoside.<sup>2-4</sup> Rutin and other flavonoids have high antioxidant properties observed

in *in vivo* and *in vitro* studies.<sup>3,5-7</sup> Furthermore, rutin is a non-toxic and non-oxidizing molecule, and it is not a pro-oxidant like myricetin and quercetin.<sup>2,8</sup> Rutin with different biological activities such as anti-inflammatory is used for treating various diseases.<sup>3</sup>

Recently, "*in situ* gel" as a newly developed drug delivery, extensively used in the drug delivery system area. Specific polymers that undergo sol-gel phase transition by induction of ambient conditions such as pH,<sup>4</sup> specific ions,<sup>5</sup> and temperature<sup>6</sup> are used in the preparation of *in situ* gels. *In situ* gel formulations are solutions or suspensions that become gels after application and thus are more acceptable to patients.<sup>7</sup> Studies have demonstrated that dermal contact times of some *in situ* gel

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systems can be up to several hours and different polymer or polymeric combinations have been used successfully to adjust the desired release profile.<sup>9</sup>

Pluronic (poloxamer) with thermoresponsive structure widely used *in situ* gel system. These polymers exhibit amphiphilic behavior because of the hydrophilic ethylene oxide and hydrophobic propylene oxide areas. The gelation of poloxamers can be described by the observed changes in the micellar structure depending on temperature and concentration. Poloxamers with sustained drug release capability have been extensively used as drug delivery systems. On the other hand, an essential handicap of poloxamers is insufficient mucoadhesive activity, thus, some poloxamer-based drug delivery formulations have been ameliorated by the addition of polymers providing mucoadhesive properties such as, sodium hyaluronate, chitosan (CH), and carbopol.<sup>10</sup> CH exhibits good properties for dermal application since it is a cationic, biocompatible, and biodegradable polysaccharide. In addition, CH has mucosal adhesion properties and good antibacterial activity.<sup>11</sup>

Cyclodextrins (CD) are generally preferred to increase the water solubility of drugs with low water solubility and low dermal permeability. It has been determined that rutin-CD inclusion complexes are formed using different CD derivatives; nevertheless, there is no *in situ* gel formulation containing rutin/sulfobutyl ether- $\beta$ -cyclodextrin (SBE- $\beta$ -CD) inclusion complexes. When the literature was examined, it was determined that SBE- $\beta$ -CD has higher complexation efficiency (EC) and higher solubility capacity than other  $\beta$ -CD derivatives.<sup>12,13</sup> Thus, it is preferred to develop drug delivery systems to increase permeability and drug solubility and accordingly enhance bioavailability in consequence.<sup>14</sup> It provides positive therapeutic effects and unimportant side effects. Therefore, it is a desirable selection for formulations of drugs with low solubilities.

The main objective of the current research is to increase the solubility of rutin with CD and placing the resulting complex in the *in situ* gel. Therefore, SBE- $\beta$ -CAD was used for this purpose. *In situ* gels containing different ratios of pluronic F-127 (PF 127) (15% - 20% - 25%) with constant concentration of CH were prepared. Additionally, developed gel formulations were evaluated in terms of clarity, pH, gelation temperature, and viscosity, and an optimum formulation was selected. For the selected formulation, 0.5% rutin and 0.5% SBE- $\beta$ -CD-rutin inclusion complex were added to *in situ* gelling systems and rutin release was assessed using *in situ* gel systems designed for dermal delivery.

## MATERIALS AND METHODS

### Materials

Rutin, PF 127, CH (low molecular weight), SBE- $\beta$ -CD, phosphate buffered saline tablets, and high performance liquid chromatography (HPLC) grade acetonitrile, and methanol were purchased from Sigma, Steinheim, Germany.

### Production of cyclodextrin-drug complex

#### Cyclodextrin-drug phase-solubility studies

Lofton and Brewster's technique<sup>15</sup> was used to conduct phase-solubility studies.

Increasing concentrations (0-10 mM) of SBE- $\beta$ -CD solution were added into a fixed amount of rutin. The resulting mixture was stirred at room temperature for 7 days with a magnetic stirrer. A 0.22  $\mu$ m membrane filter was used to filter each sample. The concentration of rutin in the supernatant was determined by an HPLC method. HPLC (Thermo Scientific, USA) analysis was carried out with a C-18 column (250 mm x 4.6 mm, 5  $\mu$ m) at a 1 mL/min flow rate and a mobile phase made up from water:methanol:acetonitrile with a 50:25:25 (v/v/v) ratio. Detection wavelength for rutin was 280 nm and the injection volume of the sample was 10  $\mu$ L, while the column temperature was held constant at 25°C. There were 3 sets of experiments (n: 3). The phase-solubility diagram was depicted by presenting SBE- $\beta$ -CD concentration against the dissolved rutin amount.<sup>16</sup>

Moreover, Higuchi and Connors classification,<sup>17</sup> that comprises AP, AL, AN, BS, and BI diagram models, was used to determine the type of diagram obtained. The equations described below were used to estimate the complex stability constant (KS) (equation 1) and EC (equation 2).<sup>15</sup>

Equation 1:

$$\text{Complex stability constant} = \frac{\text{Slope}}{50(1-\text{slope})}$$

Intrinsic rutin solubility is shown as S, which is 0.38 mM, and the linear regression's slope of the phase-solubility diagram is presented as slope.

Equation 2:

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

### Production of cyclodextrin-drug complex

The complexes were produced by kneading and freeze-drying. Thus, the efficiency of the preparation method on complexing was evaluated.<sup>18</sup>

#### Kneading

Equimolar (EqM) amounts of rutin and SBE- $\beta$ -CD were used. CD and rutin were mixed with ethanol/water (3:1 v/v) in a mortar and kneaded for 45 min. The resultant mass was stored at room temperature overnight and then dried, afterwards, the solvent was removed under reduced pressure (hot air oven, Nuve, FN 055/120, Holland) at 25  $\pm$  1°C.

#### Freeze drying

Freeze-drying is another method used to prepare CD-drug complexes.<sup>19</sup> In short, SBE- $\beta$ -CD was dissolved in water and rutin was dissolved in ethanol (EqM rate, 1:1). Then, rutin solution was transferred dropwise to the SBE- $\beta$ -CD solution and mixed with a magnetic stirrer for 24 hours. Ethanol was removed

by an evaporator and the product was lyophilized. Successful preparation of the inclusion complex was determined by differential scanning calorimetry (DSC) and fourier transform infrared spectroscopy (FTIR).

#### Production of *in situ* gel

A modified cold approach was used to produce *in situ* gelling formulations.<sup>20</sup> PF 127 solutions (15, 20, and 25% w/v) were prepared by dissolving the polymer in water (at 4°C) and a 6 phosphate buffer (at 4°C). Solutions were stored in a fridge for at least 24 h to achieve thorough dissolution. Gelling system PF 127 was contained a CH solution (1% w/v) as a mucoadhesive substance. All the samples were kept at a constant 4°C until usage. The contents of generated *in situ* gelling formulations were demonstrated in Table 1.

#### Characterization of *in situ* gel formulations

Different concentrations of PF 127 were evaluated for gelation temperature, viscosity, and pH to determine the suitability of the formulations for use as *in situ* gelling systems (Table 2).

#### pH

pH measurements were made with a pH Meter (Mettler Toledo) at 25°C, and each measurement was performed in triplicate.

#### Gelation temperature

The cold sample solution (10 mL) was heated at 2°C/min while mixed at 100 rpm on a magnetic stirrer (Thermomac-TM19). The temperature at which the magnetic stirring bar stopped moving was determined as the gelling temperature. Each measurement was performed thrice.

#### Rheological studies

The viscosity of *in situ* gels was measured using a Brookfield, DV2T-RV Viscometer (Essex, UK) with a CP 52 spindle at 10 rpm. The experiment was performed in triplicate.

#### Production of rutin-loaded *in situ* gelling systems

Rutin and rutin-SBE inclusion complex were added to the selected *in situ* gelling formulations, considering the pH,

gelation temperatures, and viscosities of all formulations. These formulations were named as SV and NZ, respectively. According to the earlier research findings, rutin has an anti-inflammatory effect when used at 500 mg. Therefore, the drug concentration in our formulations was also selected to be as 0.5%. Rutin is combined with pre-optimized but freshly prepared *in situ* gelling systems. The formulations containing rutin registered in the literature and their physical structures are given in Table 3.

#### *In vitro* release studies

The dialysis bag approach was used to undertake *in vitro* release tests with the *in situ* gel formulations.<sup>21,22</sup> The dialysis bags were filled with 100 µL formulations stored at 4°C, then 25 mL of pH 7.4 isotonic phosphate buffer at 37°C was used to immerse the dialysis bags, which had been hermetically sealed. In this way, the sink condition is provided. Each time an aliquot of the medium was withdrawn (at 15, 30, 60, 90, 180, 240, and 360 min), equivalent quantities of fresh buffer media were added to replace the withdrawn samples, and the sampling was repeated. HPLC was used to assess drug concentrations in the withdrawn isotonic phosphate buffer solutions at a pH of 7.4. The rutin release profile was depicted according to the total quantity of the drug released from each formulation over time. Each measurement was repeated three times.

## RESULTS AND DISCUSSION

#### Stability of cyclodextrin-drug complex

Phase-solubility studies are usually the preferred method for the determination of the efficacy of CD drug complexation on drug solubility.<sup>15</sup> CD/drug (1:1) inclusion complex is the most extensive type of association, where one drug molecule is incorporated into the cavity of a CD molecule, with a KS K1:1 for the equilibrium between free and associated species. When the solubility diagrams were examined, it was determined that SBE-β-CD concentration and solubility of the drug showed a linear increase (Figure 1). According to the phase-solubility diagram, it was decided to classify the SBE-β-CD diagram as "AL-type".

**Table 1. Components of *in situ* gelling formulation**

Formulation components	E1	E2	E3
PF 127 (%w/v)	25	20	15
CH (1% w/v) mL	10	10	10
Water <i>q.s.</i> to mL	100	100	100

PF 127: Pluronic F127, CH: Chitosan

**Table 2. Characterization of *in situ* gel formulations**

Formulation	pH (± SD)	Gelation temperature (°C ± SD)	Viscosity (Pa.s) 25°C
E1	6.0 ± 0.01	20 ± 1.7	168
E2	6.1 ± 0.01	25 ± 1.6	109
E3	6.1 ± 0.01	34 ± 1.1	42

(n: 3), SD: Standard deviation

**Table 3. Physical properties of drug-containing formulations and their component**

Formulation ingredients and physical properties	SV	NZ
Rutin (% w/v)	0.5	-
Rutin-SBE cyclodextrin (% w/v)	-	0.5
PF 127	15	15
CH (%1 w/v)	10	10
pH (± SD)	6.0 ± 0.01	6.1 ± 0.02
Gelation temperature (°C ± SD)	34 ± 0.9	34 ± 1.3
Viscosity (Pa.s) 25°C	42 ± 1.3	41 ± 1.7
Viscosity (Pa.s) 35°C	159.8 ± 3.4	160.7 ± 3.8

(n: 3), PF 127: Pluronic F127, CH: Chitosan, SD: Standard deviation, SBE: Sulfobutyl ether

By examining the straight line of SBE- $\beta$ -CD ( $r^2$ : 0.9196) (Figure 1), the slope was calculated as 0.5911.

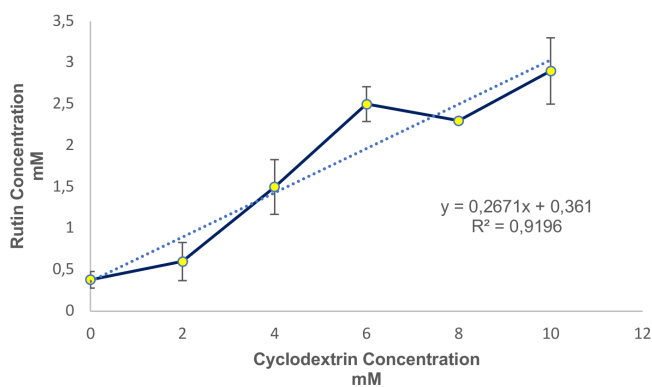
EC and KS were calculated as 0.36 and 9590 M<sup>-1</sup>, respectively. When the literature data is examined, the KSs reported were found between 100 and 10.000 M<sup>-1</sup> as the ideal value for formation of the drug:CD complex. However, with AL type solubility curve determined by examining the diagram, the drug:CD complex ratio was decided to be 1 mM:1 mM.<sup>23</sup>

DSC results of rutin, SBE- $\beta$ -CD, physical mixture (rutin-Phy), inclusion complex produced by kneading method (rutin-Knd), and inclusion complex produced by the freeze drying method (rutin-Fdy) are shown in Figure 2. The thermogram of rutin indicates two endothermic peaks at 138°C and 215°C. However, these values were found to be compatible with the literature.<sup>24</sup> When rutin-Phy and rutin-Knd were examined, it was seen that the specific peak of rutin does not disappear in these samples, but the specific peak disappears in the rutin-Fdy sample.<sup>24</sup>

When rutin FTIR result was examined, maximum peak was seen at 3277 cm<sup>-1</sup>, which was due to the hydrogen bond formed by -OH groups. The band seen at 1654 cm<sup>-1</sup>, belonged to the stretching vibration of C=O. The bands at 1601 and 1505 cm<sup>-1</sup> can be assigned to the aromatic ring vibrations of C=C. The stretching vibration of 3'-OH and 4'-OH appeared at 1456 cm<sup>-1</sup>. However, these values were found to be compatible with the literature.<sup>24</sup> When rutin-Phy and rutin-Knd spectrums were examined, it was seen that the specific peaks of rutin did not disappear; however, when rutin-fyd spectrum was examined, the specific peaks of rutin was seen to disappear (Figure 3). Also, the peaks of SBE- $\beta$ -CD were preserved, indicating the successful formation of the SBE- $\beta$ -CD-rutin complex.

### Gelation temperature

When the *in situ* gel formulations were examined, the gelation temperatures of E1 and E2 at 20°C and 25°C, respectively, were found to be low, and the gelation temperature increased with the decrease of PF 127 concentration by 15%. The gelation temperature of the E3 formulation was 34°C. In an aqueous environment, different molecules are formed in pluronics at temperatures below the critical



**Figure 1.** Phase-solubility diagram of rutin at the increasing SBE- $\beta$ -CD concentration

SBE- $\beta$ -CD: Sulfobutyl ether- $\beta$ -cyclodextrin

micelle temperature (CMT), at which the critical micelle concentration occurs. Additionally, above CMT, all individual molecules are forced to form micelles, which surround the hydrophobic core by hydrophilic chains of pluronic facing the aqueous medium. CMT values are inversely proportional to the pluronic concentration.<sup>22</sup> This inverse ratio affects the gelling temperature as the gelling temperature is highly dependent on the concentration of pluronics. While they form monomolecular micelles at lower concentration, a multimolecular lattice structure is observed at higher concentration.<sup>23</sup> Due to this temperature phenomenon, it was tried bringing the gelling temperature closer to the skin temperature in the experimental formulations. Therefore, PF 127 concentrations were reduced. In the E1-E3 formulations, PF127 concentrations were decreased to increase the gelation temperature. Similar findings were observed in other studies.<sup>25</sup>

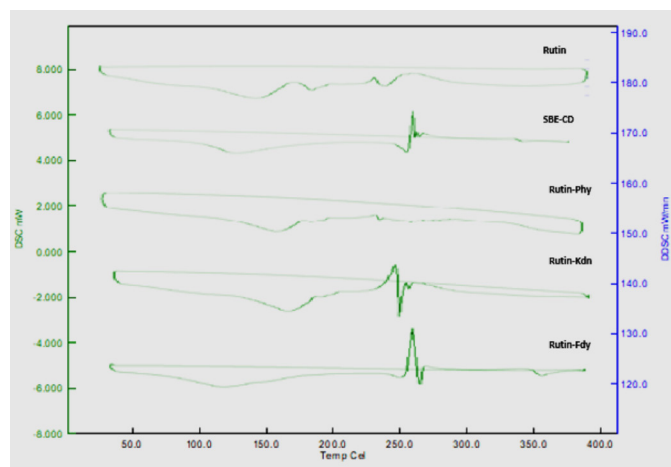
### pH

pH is a significant parameter in dermal formulations. Physiological pH in healthy skin and 5.5 on average. The pH of dermal carriers is a significant parameter as the change in pH of the skin will cause unwanted effects such as rash and itchiness.<sup>21</sup> pH of all formulations were between 6.0-6.1 as presented in Table 2.

Since CH (aq) is obtained by dispersing it in a sufficient amount of acetic acid (aq) (1% w/v), the pH of the formulations containing CH was determined as 6.0-6.1. This result supports the data we found in the literature review.<sup>26</sup>

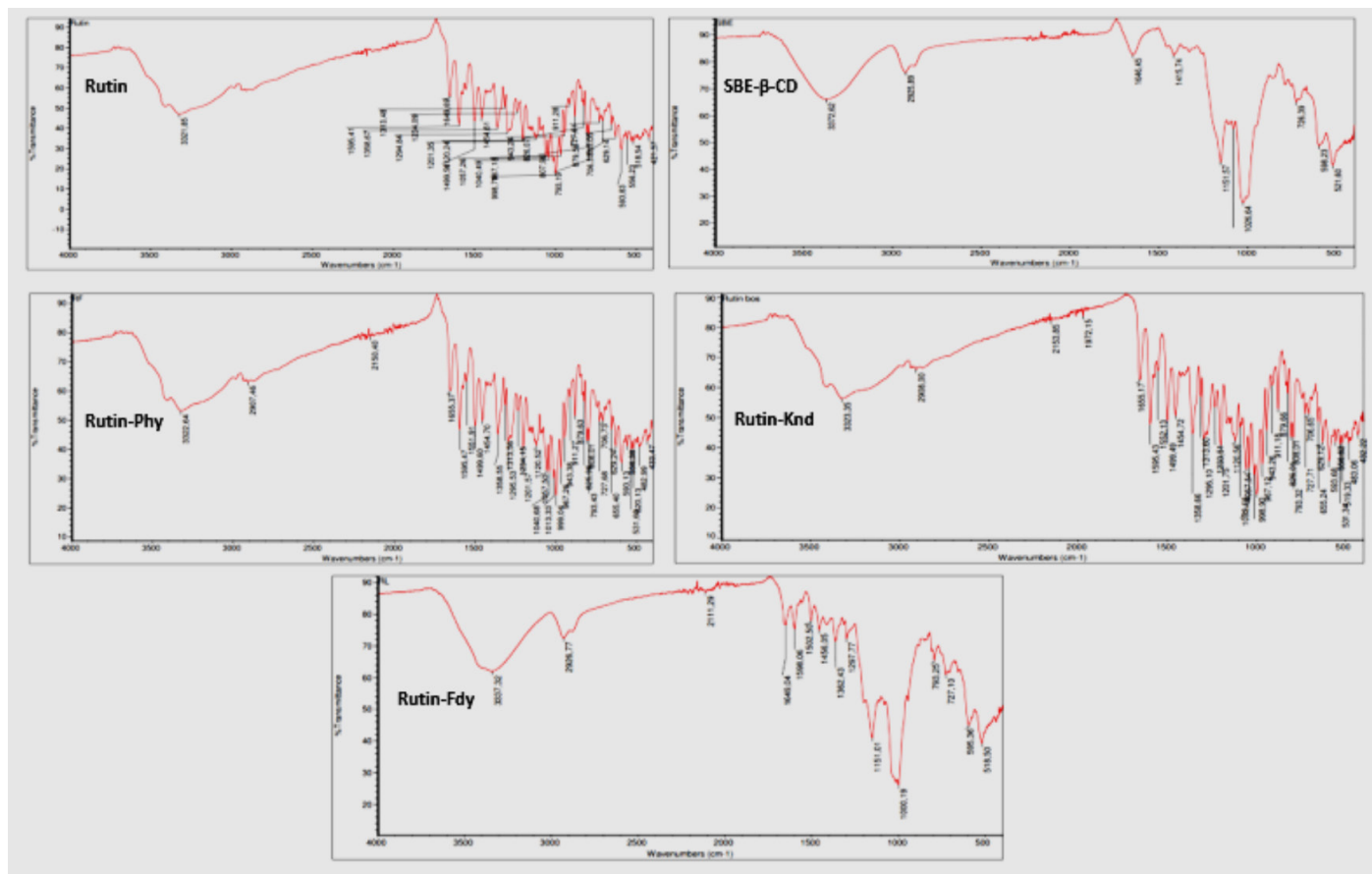
### Viscosity

Viscosity of the gels was increased (from 38 to 165 Pa.s at 20°C) by increasing the concentration of PF 127 (from 15% to 25%). It indicated that PF 127 concentration extremely affects the viscosity of the gels. After characterization, the optimum formulation was selected in terms of pH value, gelation temperature, and viscosity. The optimized formulation contained



**Figure 2.** DSC thermogram of rutin, SBE- $\beta$ -CD, rutin-Phy, rutin-Knd and rutin-Fdy

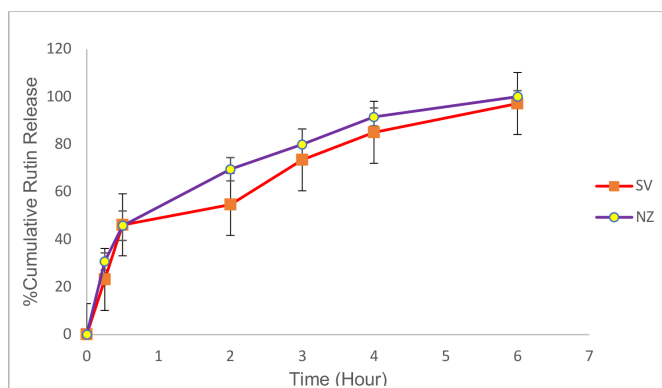
SBE- $\beta$ -CD: Sulfobutyl ether- $\beta$ -cyclodextrin, DSC: Differential scanning calorimetry



**Figure 3.** FTIR spectra of rutin, SBE- $\beta$ -CD, rutin-Phy, rutin-Knd and rutin-Fdy  
SBE- $\beta$ -CD: Sulfolbutyl ether- $\beta$ -cyclodextrin, FTIR: Fourier transform infrared spectroscopy

rutin (0.5% w/v), rutin-SBE- $\beta$ -CD inclusion complex (0.5% w/v) in the *in situ* gelling formulations, chosen to be suitable.

These formulations are shown in Table 3 as SV and NZ. pH of the formulations was between 5.9 and 6.0 with gelling temperatures at 34°C. Also, release of the in the NZ formulations, during the first two hours, 57% of the drug was released and the release was continued up to 77% by the end of three hours and it may be due to the concentration of PF 127 and CH. Whereas SV formulations showed <57% drug release after two hours (Figure 4). This could be due to the usage of CDs in the NZ formulation by the investigation of *in vitro* release studies results, it was found that the BRN formulation exhibits faster drug release than NI formulation. This was due to Rut-SBE-CD inclusion complex in the BRN formulation. It is believed that the CD complex caused increasing solubility of the drug. An identical result was obtained in another study, which was conducted by Polat et al.<sup>27</sup> They produced insert formulations containing besifloxacin HCL and besifloxacin HCl-CD inclusion complex. The results indicated that the release rate of the insert formulation containing the complex was higher than the formulation containing only the drug.<sup>27</sup>



**Figure 4.** *In vitro* release profiles of *in situ* gel formulation

## CONCLUSION

In this study, SBE- $\beta$ -CD was used. SBE- $\beta$ -CD was found to increase the solubility of rutin by 8 times. Inclusion complexes of drug-SBE- $\beta$ -CD were produced with different methods. Due to DSC and FTIR studies, it was determined that the production was successfully carried out *via* the freeze-drying method. Furthermore, it has been determined that the prepared *in situ* gels have the optimum gelling temperature, gelling

capacity, appropriate pH point, and desired properties such as great appearance. The formulations were observed to have pseudoplastic behavior, pH: 6, and gelation temperatures between 22°C and 34°C. *In vitro* release studies have revealed that rutin both increases in solubility and dissolves over a longer period of 6 h. Rutin is a common anti-inflammatory natural compound effective against many diseases. The selected formulations contained 0.5% w/v. The *in situ* gel contact time with the skin is prolonged and it could provide drug release for a longer time.

#### Ethics

**Ethics Committee Approval:** Not applicable.

**Informed Consent:** Not applicable.

**Peer-review:** Externally peer-reviewed.

#### Authorship Contributions

Surgical and Medical Practices: S.G., K.H.P., Concept: S.G., K.H.P., Design: S.G., K.H.P., Data Collection or Processing: S.G., K.H.P., Analysis or Interpretation: S.G., K.H.P., Literature Search: S.G., K.H.P., Writing: S.G., K.H.P.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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