Original Article
DOI: 10.4274/tjps.galenos.2022.00334

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

Short Title: Rutin for Dermal Applications

Sefa Gözcü[1], Kerem Heybet Polat[2]
[1]Department of Pharmacognosy, Faculty of Pharmacy, Erzincan Binali Yıldırım University, 24002, Erzincan, Turkey
[2]Department of Pharmaceutical Technology, Faculty of Pharmacy, Erzincan Binali Yıldırım University, 24002, Erzincan, Turkey

Corresponding Author Information
Sefa Gözcü
sgozcu@erzincan.edu.tr
https://orcid.org/0000-0002-0735-4229
+90 446 224 53 44
18.01.2022
29.04.2022
05.05.2022

Abstract
Objective: Rutin has been broadly applied in the treatment of several diseases due to its pharmacological activities. However, its low aqueous solubility limits its absorption and bioavailability. The aim of this research is 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 SBE-*β*-CD. Rutin- SBE-*β*-CD inclusion complex was prepared by kneading and freeze dying method. Structural characterization was carried out using DSC and FTIR. İn 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 like pH, clarity, gelation temperature and viscosity were determined.
Results: When the solubility diagrams were examined, it was concluded that SBE-*β* 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 which were between 20 °C to 34 °C. For the selected formulation(Formulation E3) 0.5% Rutin and Rutin/ SBE-*β*-CD were transfered to *in situ* gelling system. As a result of *in vitro* release studies, it was observed that the release of the Rutin/SBE-β-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-*β*-CD.
Key Words
Rutin, In situ gel, Dermal Drug Delivery Systems, SBE-*β*-cyclodextrin, Pluronic

1.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 been found to have many pharmacological activities, such as anticancer, anti-inflammatory, antioxidant, hypoglycemic, diuretic, and hepatoprotective [1].

Rutin (quercetin-3-*O*-Rutinoside) is in the form of flavonol glycosides, and is also known as, vitamin P and has a structure of 5,7,30,40-tetrahydroxy flavone-3-rhamno glucoside [2-4]. Rutin and other flavonoids have been reported to have high antioxidant properties observed in *in vivo* and *in vitro* studies [3, 5-7]. Furthermore, Rutin is a non-toxic and non-oxidizing molecule, and it is not a pro-oxidant like myricetin and quercetin [2, 8]Rutin with different biological activities (like anti-inflammatory) is used in the treatment of various diseases[3].

In recent years, "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 [4], specific ions[5] and temperature [6] are used in the preparation of in situ gelsIn-situ gel formulations are solutions or suspensions that become gels after application and thus are more acceptable to patients.[7]. Studies have demonstrated that dermal contact times of some in situ gel systems can be up to several hours and different polymer or polymeric combinations have been used successfully to adjust the desired release profile [9]. pluronics (polaxamer) with thermoresponsive structure widely utilized in situ gel system. These polymers exhibit amphiphilic behavior because of the hydrophilic ethylene oxide and hydrophobic propylene oxide area. The gelation of poloxamers can be described by the observed changes in 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 Poloxamer is insufficient mucoadhesive activity; thus, some Poloxamer-based drug delivery formulation have been ameliorated by the addition of polymers providing mucoadhesive property such as, sodium hyaluronate, chitosan and carbopol [10]. Chitosan (CH) exhibits good properties for dermal application since it is a cationic, biocompatible, and biodegradable polysaccharideIn addition, chitosan has been shown to have mucosal adhesion properties and good antibacterial activity[11].

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 by using different CD derivatives nevertheless, there is no in situ gel formulation containing Rutin/sulfobutyl ether-*β*-cyclodextrin (SBE-β-CD) inclusion complexes. When the literature was examined, it was determined that SBE-*β*-CD has higher complexation efficiency and higher solubility capacity than other *β*-CD derivatives [12, 13]. Thus, It is preferred to develop drug delivery systems to increase permeability and drug solubility and accordingly enhance bioavailability. in consequence 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, SHE β-CAD was utilized 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. In addition, developed gel formulations were evaluated in terms of clarity, pH, gelation temperature, and viscosity, and a optimum formulation was selected.For the selected formulation, 0.5% Rutin and 0.5% SBE-β-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

2.1 Materials

Rutin, PF 127 , chitosan (low molecular weight), SBE-*β*-CD, phosphate buffered saline (PBS) tablets and HPLC grade acetonitrile (ACN) and methanol were purchased from Sigma, Steinheim, Germany.

2.2. Production of cyclodextrin-drug complex

2.2.1. Cyclodextrin-drug phase-solubility studies

Loftson and Brewster's technique [15] was utilized to conduct phase-solubility studies. Increasing concentrations (0-10 mM) of SBE-β-CD solution was added into a fixed amount of Rutin. The resulting mixture was stirred at room temperature for seven days with a magnetic stirrer. A 0.22 µm membrane filter was used to filter each sample. The concentration of Rutin in the supernatant was was determined by HPLC method. The HPLC (Thermo Scientific, USA) analysis was carried out with a C-18 column (250 mm x 4.6 mm, 5 mm) at a 1 mL/min flow rate and a mobile phase made up from Water:Methanol:Acetonitril with a 50:25:25 (v/v/v) ratio. Rutin's detection wavelength was 280 nm, and the injection volume of the sample was 10 µL, while column temperature was held constant at 25 °C. There were three sets of experiments (n=3). The phase-solubility diagram was depicted by presenting SBE-*β*-CD concentration against the dissolved Rutin amount [16].

Moreover, Higuchi and Connors classification [17], 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 (Eq. 1) and complexation efficacy (Eq. 2) [15].

Eq. 1:

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

intrinsic Rutin solubilitys is shown as S, which is 0.38 mM, and the linear regression's slope of the phase-solubility diagram is showed as Slope.

Eq. 2:

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

2.3.2. 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 [18].

Kneading

Equimolar (EqM) amounts of Rutin and SBE-β-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, the solvent was removed under reduced pressure (hot air oven, Nuve, FN 055/120,Holland) at 25 ± 1°C.

Freeze drying

Freeze-drying is another method used to prepare CD-drug complexes [19]. In short, SBE-*β*-CD were dissolved in water and Rutin was dissolved in ethanol (equimolar rate, 1:1). Then, Rutin solution was transfered dropwise to the SBE-*β*-CD solution and mixed with a magnetic stirrer for 24 hours. Ethanol was removed by an evaporator and the product is lyophilized. Successful preparation of the inclusion complex was determined by DSC and FTIR.

2.4 Production of in situ gel

A modified cold approach was used to produce in situ gelling formulations [20]. PF127 solutions (15, 20, and 25% w/v) were prepared by dissolving the polymer in water (at 4 °C) and a pH 6 phosphate buffer (at 4 °C). Solutions were stored in a fridge for at least 24 hours to achieve thorough dissolution. Gelling system PF127 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.

2.5. Characterization of in situ gel formulations

Different concentrations of PF127 were evaluated for gelation temperature, viscosity and pH to determine the suitability of the formulations for use as in situ gelling systems (Table **2**).

2.6. pH

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

2.7. 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 three times.

2.8. Rheological Studies

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

2.9. 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 SV and NZ, respectively. According to the earlier researh findings, Rutin shows an anti-inflammatory effect when it is 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.

2.10. *In Vitro* Release Studies

The dialysis bag approach was used to undertake *in vitro* release tests with the in situ gel formulations [21, 22]. The dialysis bags were filled with 100 µL of 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, 240,360 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. Rutin release profile was depicted according to the total quantity of drug released from each formulation over time. Each measurement was repeated three times (iş it the measurement of the same sample or repating the whole experiment 3 times).

3.Results and Discussion

3.1.Stability of cyclodextrin-drug complex

Phase-solubility studies are usually the preferred method for determination of the efficacy of CD drug complexation on drug solubility [15]. The 1:1 CD/drug inclusion complex is the most extensive type of association where one 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 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”. By examining the straight line of SBE-*β*-CD (r^2 = 0,9196) (Fig. 1), the slope was calculated as

0,5911. Complexation efficiency (EC) and stability constant (KS) were calculated as 0,36 and 9590 M^{-1}, respectively. When the literature data is examined the stability constants reported were found were between 100 and 10,000 M^{-1} as the ideal value for the formation of the drug:CD complex. However, with the AL type solubility curve determined by examining the diagram, the drug:CD complex ratio was decided to be 1 mM:1 mM [23,29].

DSC results of Rutin, SBE-*β*-CD, physical mixture (Rutin Phy), inclusion complex produced by kneading method (Rutin-Knd) and inclusion complex produced by freeze dying method (Rutin-Fdy) are shown in Figure 2. Thermogram of Rutin indicates two endothermic peaks at 138°C and 215°C. However, these values were found to be compatible with the literature [30].When the Rutin-Phy,and Rutin-Knd were examined, it was seen that the specific peak of Rutin does not disappear ın these samples, but the specific peak disappears in the Rutin-Fdy sample [30].

When Rutin FTIR result was examined, maximum peak seen at 3277 cm^{-1} which was due to the hydrogen bond formed by –OH groups. The band seen at 1654 cm^{-1} , belonged to the stretching vibration of C=O. The 1601 and 1505 cm^{-1} can be assigned to the aromatic ring vibrations of C=C. The stretching vibration of 3'-OH and 4'-OH appears at 1456 cm^{-1} . However, these values were found to be compatible with the literature [30]. 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, it was seen that the specific peaks of Rutin disappeared (Fig 3).Also, the peaks of SBE-*β*-CD were preserved, indicating the successful formation of the SBE-*β*-CD-Rutin complex.

3.2.Gelation Temperature

When the in situ gel formulations are examined, it was found that the gelation temperatures of E1 and E2 were low , 20°C and 25°C respectively 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 micelle temperature (CMT) at which the critical micelle concentration (CMC) occurs. In addition, above the critical micelle temperature (CMT), all individual molecules are forced to form micelles, that surround the hydrophobic core by hydrophilic chains of pluronics facing to aqueous medium. CMT values are inversely proportional to pluronic concentration [22]. 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, multimolecular lattice structure is observed at higher concentration[23]. Due to this temperature phenomenon, it was tried to bring the gelling temperature closer to the skin temperature in the experimental formulations. Therefore, PF127 concentrations were reduced. In the E1-E3 formulations, the PF127 concentrations were decreased to increase the gelation temperature. Similar findings were observed in other studies [24].

3.3.pH

pH is a significant parameter in the dermal formulation.Physiological pH in healthy skin is 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 [21]. The pH of all formulations were between 6,0-6,1 and are 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 literaturereview[25].

3.4. Viscosity

The viscosity of the gels was increased (from 38 to 165 Pa.s at 20 °C,) by increasing the concentration of PF127 (from 15% to 25%). it indicating that PF 127 concentration extrimily 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 Rutin (0.5 % w/v), Rutin-SBE β CD inclusion complex (0.5 % w/v) in the in situ gelling formulations, choosen to be suitable.

These formulations are shown in Table 3 as SV and NZ. The pH of the formulations was between 5.9-and 6.0 with gelling temperatures of 34 °C. And the viscosity of the In the NZ formulations, during the first tow hours 57% of drug was released and the release was continued upto 77% by the end of three hours. And it may be due to concentration of PF 127 and CH. Whereas, SV formulations showed <57 % drug release after two hour. This could be due to the usage of CDs in the NZ formulationby the investigation of in vitro release studies results, it was found that the BRN formulation shows faster drug release than Nl formulation.This was due to the Rut-SBE CD inclusion complex in the BRN formulationIt is believed that the CD complex caused the increasing solubility of the drug. An identical result was obtained in another study which is conducted by Polat et al. 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[26].

4.Conclusion

In this study, it was used SBE-β-CD. It was found that SBE-β-CD increased the solubility of Rutin by 8 times. 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. 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 shown that Rutin both increases in solubility and dissolves over a longer period of 6 hours. Rutin is a common anti-inflammatory drug that is used in many diseases. The selected formulations contain 0.5% w/v. The in situ gel contact time with skin is prolonged and it could provide drug release for a longer period of time

Referances

1. Nijveldt RJ, Van Nood E, Van Hoorn DE, Boelens PG, Van Norren K, Van Leeuwen PA. Flavonoids: a review of probable mechanisms of action and potential applications. Am. J. Clin. Nutr. 2001; 74:418-425.
2. Chat OA, Najar MH, Mir MA, Rather GM, Dar AA. Effects of surfactant micelles on solubilization and DPPH radical scavenging activity of Rutin. J. Colloid Interface Sci. 2011; 355:140-149.
3. Kazłowska K, Hsu T, Hou CC, Yang WC, Tsai GJ. Anti-inflammatory properties of phenolic compounds and crude extract from Porphyra dentata. J. Ethnopharmacol. 2010; 128:123-130.
4. Srividya B, Cardoza RM, Amin P. Sustained ophthalmic delivery of ofloxacin from a pH triggered in situ gelling system. J Control Release. 2001; 73:205-211.
5. Liu Z, Li J, Nie S, Liu H, Ding P, Pan W. Study of an alginate/HPMC-based in situ gelling ophthalmic delivery system for gatifloxacin. Int. J. Pharm. 2006; 315:12-17.
6. Wei G, Xu H, Ding PT, Zheng JM. Thermosetting gels with modulated gelation temperature for ophthalmic use: the rheological and gamma scintigraphic studies. J Control Release. 2002; 83:65-74.
7. Almeida H, Amaral MH, Lobão P, Lobo JMS. In situ gelling systems: a strategy to improve the bioavailability of ophthalmic pharmaceutical formulations. Drug Discov. Today. 2014; 19:400-412.
8. Almeida JS, Lima F, Da Ros S, Bulhoes LO, de Carvalho LM, Beck RC. Nanostructured systems containing Rutin: in vitro antioxidant activity and photostability studies. Nanoscale Res. Lett. 2010; 5:1603-1610.
9. Verma A, Tiwari A, Saraf S, Panda PK, Jain A, Jain SK. Emerging potential of niosomes in ocular delivery. Expert Opin Drug Deliv. 2021; 18:55-71.
10. Dumortier G, Grossiord JL, Agnely F, Chaumeil JC. A review of poloxamer 407 pharmaceutical and pharmacological characteristics. J. Pharm. Res. 2006; 23:2709-2728.
11. Muxika A, Etxabide A, Uranga J, Guerrero P, De La Caba K. Chitosan as a bioactive polymer: Processing, properties and applications. Int. J. Biol. Macromol. 2017; 105:1358-1368.
12. Aiassa V, Zoppi A, Becerra MC, Albesa I, Longhi MR. Enhanced inhibition of bacterial biofilm formation and reduced leukocyte toxicity by chloramphenicol: β-cyclodextrin: N-acetylcysteine complex. Carbohyd Polym. 2016; 152:672-678.
13. Jithan A, Mohan CK, Vimaladevi M. Development and evaluation of a chloramphenicol hypertonic ophthalmic solution. Indian J. Pharm. Sci. 2008; 70:66.
14. Zuorro A, Fidaleo M, Lavecchia R. Solubility Enhancement and Antibacterial Activity of Chloramphenicol Includedin Modified β-Cyclodextrins. Bull Korean Chem Soc. 2010; 31:3460-3462.
15. Loftsson T, Brewster ME. Pharmaceutical applications of cyclodextrins: basic science and product development. J. Pharm. Pharmacol. 2010; 62:1607-1621.
16. Williams HD, Trevaskis NL, Charman SA, Shanker RM, Charman WN, Pouton CW, Porter CJ. Strategies to address low drug solubility in discovery and development. Pharmacol. Rev. 2013; 65:315-499.
17. Higuchi T. A phase solubility technique. Adv. Anal. Chem. Instrum. 1965; 4:117-211.
18. Ribeiro A, Figueiras A, Santos D, Veiga F. Preparation and solid-state characterization of inclusion complexes formed between miconazole and methyl-β-cyclodextrin. Aaps Pharmscitech. 2008; 9:1102-1109.

19. Covre JL, Cristovam PC, Loureiro RR, Hazarbassanov RM, Campos M, Sato ÉH, Gomes JÁP. The effects of riboflavin and ultraviolet light on keratocytes cultured in vitro. Arq Bras Oftalmol. 2016; 79:180-185.
20. El-Kamel A. In vitro and in vivo evaluation of Pluronic F127-based ocular delivery system for timolol maleate. Int. J. Pharm. 2002; 241:47-55.
21. Erol I, Üstündağ Okur N, Orak D, Sipahi H, Aydın A, Özer Ö. Tazarotene-loaded in situ gels for potential management of psoriasis: biocompatibility, anti-inflammatory and analgesic effect. Pharm Dev Technol. 2020; 25:909-918.
22. Aytekin E, Öztürk N, Vural İ, Polat HK, Çakmak HB, Çalış S, Pehlivan SB. Design of ocular drug delivery platforms and in vitro-in vivo evaluation of riboflavin to the cornea by non-interventional (epi-on) technique for keratoconus treatment. J Control Release. 2020; 324:238-249.
23. Escobar-Chávez J, López-Cervantes M, Naik A, Kalia Y, Quintanar-Guerrero D, Ganem-Quintanar A. Applications of thermo-reversible pluronic F-127 gels in pharmaceutical formulations. J. Pharm. Pharm. Sci. 2006; 9:339-358.
24. Edsman K, Carlfors J, Petersson R. Rheological evaluation of poloxamer as an in situ gel for ophthalmic use. *Eur J Pharm Sci* . 1998; 6:105-112.
25. Pawar P, Kashyap H, Malhotra S, Sindhu R. Hp--CD-voriconazole in situ gelling system for ocular drug delivery: in vitro, stability, and antifungal activities assessment. Biomed Res. Int. 2013; 2013.
26. Polat HK, Pehlivan SB, Özkul C, Çalamak S, Öztürk N, Aytekin E, Fırat A, Ulubayram K, Kocabeyoğlu S, İrkeç M. Development of besifloxacin HCl loaded nanofibrous ocular inserts for the treatment of bacterial keratitis: In vitro, ex vivo and in vivo evaluation. Int. J. Pharm. 2020; 585:119552.

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 q.s. to mL	100	100	100

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

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

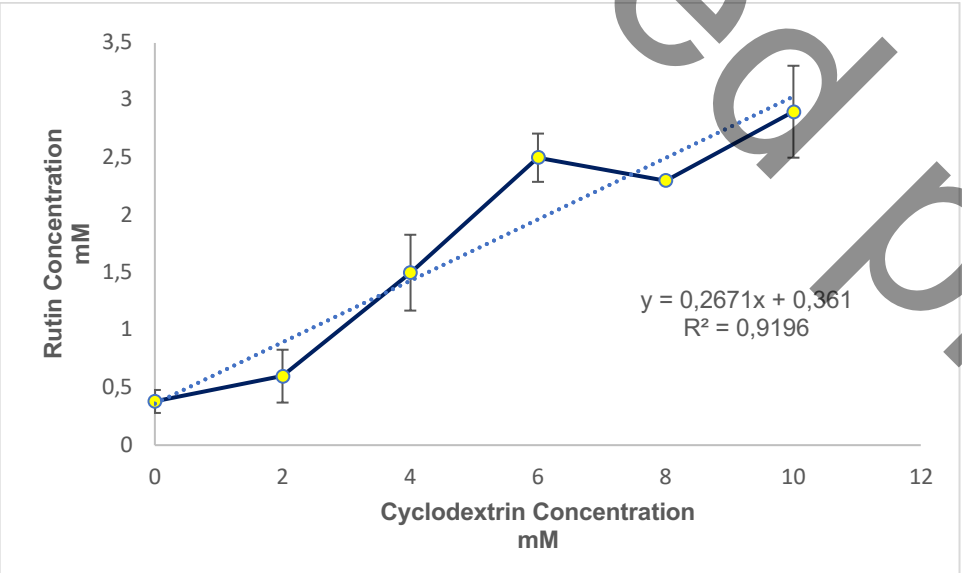

Figure 1: Phase-solubility diagram of Rutin at the increasing SBE-β-CD concentration

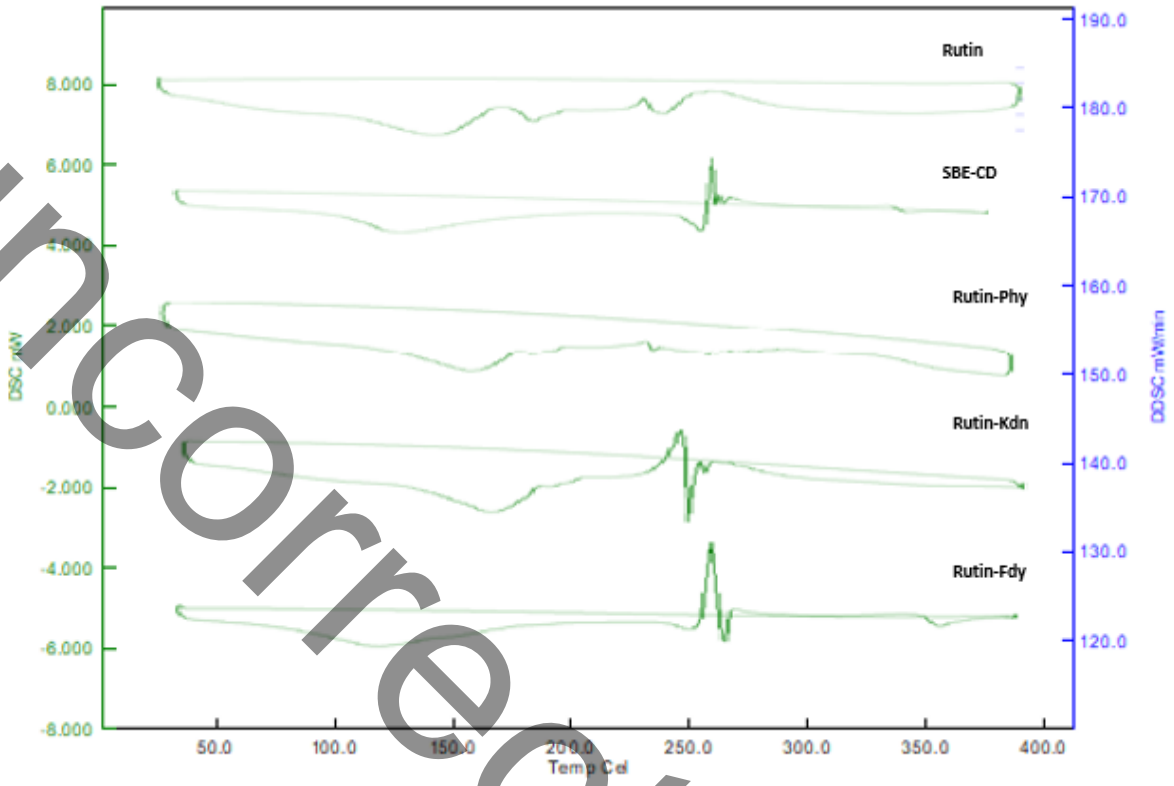

Figure 2: DSC thermogram of Rutin, SBE-β-CD, Rutin-Phy, Rutin-Knd and Rutin-Fdy

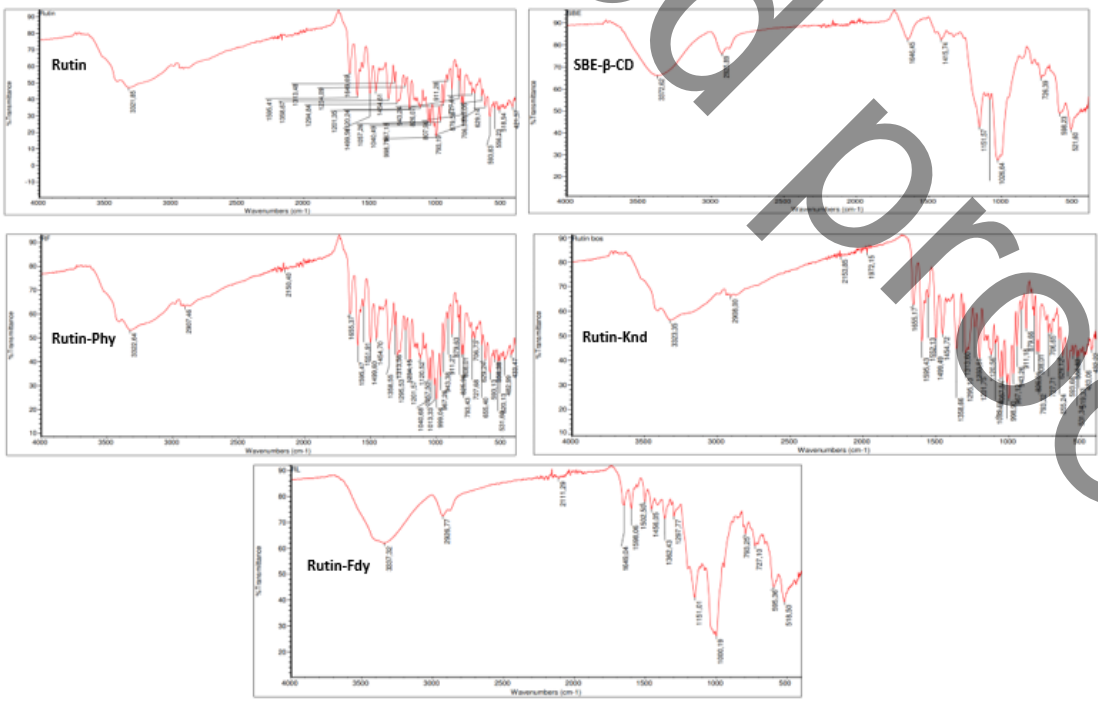

Figure 3: FTIR spectra of ruitn, SBE-β-CD, Rutin-Phy, Rutin-Knd and Rutin-Fdy

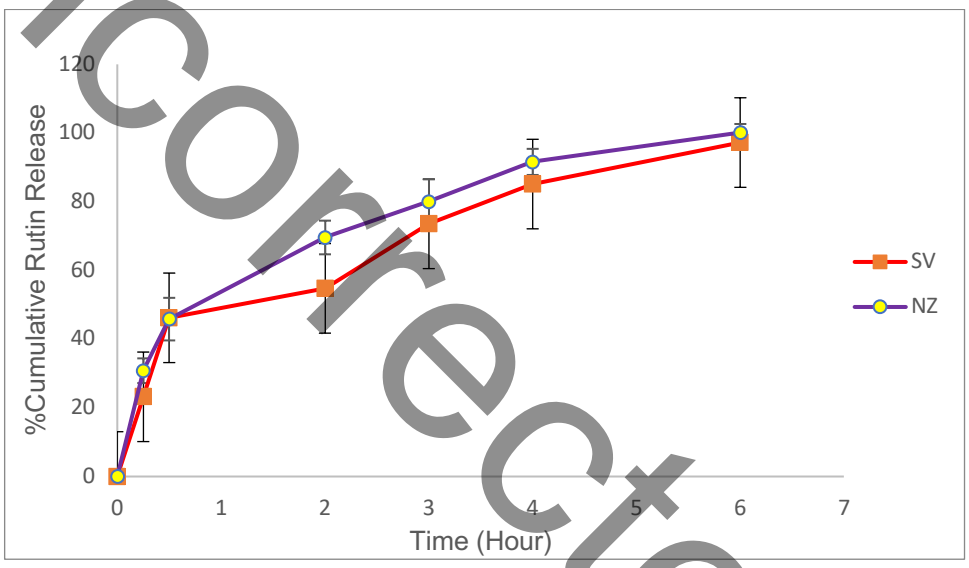

Fig.4: In vitro release profiles of in situ gel formulation