

Development and evaluation of azelaic acid-cyclodextrin hydrogels for treatment of acne

Muhammet Davut ARPA ^{1*} , Nilay DOĞAN ¹ , Melisa GÜNDÜZ ² , Elçin Su KARAVELİOĞLU ² , Ümit Can ERİM ³ 

¹ Department of Pharmaceutical Technology, Istanbul Medipol University, School of Pharmacy, 34815, Istanbul, Türkiye.

² Istanbul Medipol University, School of Pharmacy, 34815, Istanbul, Türkiye.

³ Department of Analytical Chemistry, Istanbul Medipol University, School of Pharmacy, 34815, Istanbul, Türkiye.

* Corresponding Author. E-mail: mdarpa@medipol.edu.tr (M.D.A.); Tel. +90-216 681 22 64.

Received: 26 October 2023 / Accepted: 11 December 2023

ABSTRACT: Acne is an inflammatory or non-inflammatory disease that can be seen at every stage of human life and affects daily life both physically and psychologically. In the topical treatment of acne, substances with different pharmacological effects such as benzoyl peroxide, isotretinoin, azelaic acid (AZE), and erythromycin are used alone or in combination. AZE cream (20%) has been frequently preferred in the treatment of mild and moderate acne in recent years. However, since AZE has poor solubility in water, its low permeability problems may occur. In this study, the soluble form of AZE was obtained by forming an inclusion complex of AZE with 2-hydroxypropyl β -cyclodextrin (H β C). In addition, the gel formulation, which generally exhibits higher permeability properties than oily formulations, was prepared using hydroxypropyl methylcellulose (HPMC). The formation of the inclusion complex was confirmed by NMR, XRD, DSC, and FTIR analyses. As a result of the characterization studies of the hydrogel formulations, it was determined that they had pseudoplastic flow and their viscosity was approximately 220 Pa.s. In drug release studies conducted with the dialysis bag method, it was found that the AZE-H β C hydrogel formulations had a similar cumulative release percent as H β C-free hydrogels but showed a higher cumulative release percent than the commercial cream (Azelderm, Orva, Türkiye). It was proven that the drug release of the formulations that exhibit a release profile in accordance with Higuchi kinetics was different from Azelderm according to difference (f1) and similarity (f2) factors.

KEYWORDS: Azelaic acid; acne; cyclodextrin; HPMC; hydrogel.

1. INTRODUCTION

Acne, with a lifetime prevalence of approximately 85%, is a disease of pilosebaceous unit that usually occurs as inflammatory or non-inflammatory lesions [1]. It profoundly affects people's quality of life, not only in terms of physical appearance but also because it has negative psychological and social effects [2]. Reducing acne scars and shortening treatment time are the main goals of acne treatment. Topical treatment using active substances such as AZE, benzoyl peroxide, isotretinoin, and erythromycin is a suitable option in terms of patient compliance. These active ingredients are presented in semi-solid drug carrier systems such as gel, cream, and lotion [3].

Numerous studies have been carried out with different carrier systems, like gels, as opposed to oil-based drug carriers, to improve the permeability of drugs [4]. Hydrogel is defined as three-dimensional network structures made from a group of synthetic and/or natural polymers that have a high water absorption and retention capacity [5]. A chemical substance or cell can be loaded into a hydrogel and provided controlled release from the hydrogel to medium due to the porous of the gel matrix, which is an important advantage of hydrogel [6]. Hydrogels are also very convenient and attractive for topical application of modern drug delivery systems such as liposome, microemulsion, nanocomposite, and cyclodextrin-based inclusion complex thanks to their advantages such as facilitating applicability and increasing residence time, efficiency, and patient compliance [7-10]. Cyclodextrin (CD) is a crystalline, non-hygroscopic, cyclic oligosaccharide manufactured from starch. They are water-soluble because of the large number of hydroxyl groups and while the outer coat of the torus is hydrophilic, the cavity's inner surface is hydrophobic [11,12]. With this three-

How to cite this article: Arpa MD, Doğan N, Gündüz M, Karavelioğlu MS, Erim EC. Development and evaluation of azelaic acid-cyclodextrin hydrogels for treatment of acne. J Res Pharm. 2024; 28(1): 177-190.

dimensional special structure, they form inclusion complexes to improve the water solubility of hydrophobic active ingredients [13,14]. Among the three types of CDs (α -, β -, and γ -CD), the most frequently used in the pharmaceutical field is β -CD. H β C is a modified form of naturally occurring β -CD with the addition of a hydroxypropyl group to help increase the water solubility of a drug [15].

AZE, an aliphatic dicarboxylic acid, is one of the most effective agents in treating mild to moderate acne and has similar efficacy to other well-known treatments such as benzoyl peroxide, erythromycin, and tretinoin [16]. It has been demonstrated that the cream formulation of AZE (20%) is effective against many pathogenic conditions of acne, as it has anti-inflammatory, antibacterial, anti-keratinizing, antioxidant, and tyrosinase-inhibiting effects [17,18]. However, the water solubility of this natural compound is quite low [19], which may cause poor penetration through the skin. As mentioned above, CDs can provide opportunities as a very attractive agent in this respect. In the literature, CD and its derivatives are used safely in high amounts to increase the solubility of hydrophobic active substances [20,21]. Moreover, they have been used in the development of topical formulations of active substances such as isotretinoin [22] and tretinoin [23] in the treatment of acne. There are studies on preparing CD inclusion complexes [24] and nanosponge [25] to increase the solubility of AZE. However, no study was found investigating the effectiveness of the AZE-H β C inclusion complex as a semi-solid drug formulation.

In this study, the AZE-H β C inclusion complex was investigated and then hydrogel formulations were prepared with the addition of HPMC. Characterization studies such as NMR, XRD, etc. of the AZE-H β C complex were performed and the viscosity, pH, mechanical properties, and bioadhesive capability of the hydrogels were determined. *In vitro* drug release studies by dialysis bag were carried out to compare the release properties with commercial cream and classic gels.

2. RESULTS AND DISCUSSION

2.1. Preparation of AZE- H β C inclusion complex

The minimum molar ratio of H β C required to dissolve AZE was determined. For this purpose, the solubility was checked visually by adding H β C between 0.17 and 2 M ratio to dissolve 1 M of AZE. It was observed that 1.17 M of H β C (C7) was sufficient to dissolve AZE (Table 4). However, as the amount of added H β C increased, AZE began to clump together with the increase in viscosity and did not dissolve completely. High viscosity is one of the factors that negatively affect the solubility of solid matter [26,27]. The amount of dissolved AZE was also analyzed by HPLC (data not shown). Accordingly, it was confirmed by the HPLC analysis that the solubility increased as the amount of H β C increased in the molar ratio between 0.17–1.17 but the amount of dissolved AZE decreased when used at the higher molar ratio of H β C. The results presented that approx. 4 times higher solubility was obtained with the C7 sample than pure AZE. While insoluble AZE particles were observed in the C10 formulation, C11 could not be analyzed by HPLC due to its high viscosity.

Although AZE has poor water solubility at 20–25°C [19,28], its solubility increases in warm-hot water [29,30]. CDs with a cyclic oligosaccharide structure improve the aqueous solubility of substances with occurring three-dimensional inclusion complex thanks to hydrophobic internal cavities and a hydrophilic external surface [31,32]. To increase the solubility of AZE, Manosroi *et al.* [24] prepared a physical mixture of AZE and H β C and their inclusion complexes by co-evaporation and freeze-drying method (at a molar ratio of 1:1). As a result, they found that the use of CD increased the solubility of AZE with each method. Kumar and Rao [25] showed that the solubility of AZE (1 M) in water was approximately 3 times higher than pure AZE, with the use of 1 M of H β C. Our findings were consistent with the literature.

2.2. Characterization of AZE-H β C complex

When AZE is involved in an inclusion complex along with CD, which has an amorphous structure, no crystal structure remains in order to absorb the energy in the environment. Therefore, the endothermic melting peak of this inclusion complex disappears from its thermogram [33]. DSC thermogram of the materials is demonstrated in Figure 1a. The characteristic endothermic peak detected at 112.46 °C of the DSC thermogram of AZE indicated the melting point. The small endothermic peaks observed at 204° C of the H β C thermogram and at 270 °C AZE-H β C thermogram demonstrated that AZE and H β C peaks disappeared. In addition, these findings suggested that AZE located in the gaps found in the amorphous structure of H β C and involved in the amorphous structure [24,25,33].

X-ray diffraction diffractogram is among the procedures used to confirm the formation of inclusion complex since AZE has a crystal structure, whereas the H β C has an amorphous structure. X-ray diffractogram of AZE given in Figure 1b demonstrated specific peaks at 19.26° (3365), 23.06° (6066), 27.3° (1964), 28.34° 2 θ

angles, which confirmed its crystal structure. On the other hand, no sharp peaks were observed at the X-ray diffractogram of H β C due to its amorphous structure. No sharp and specific peak was detected at the X-ray diffractogram of the AZE-H β C inclusion complex, which overall confirmed the formation of the inclusion complex [24,25].

In order to define an inclusion complex, ^1H NMR spectra have been widely used. When AZE located at the gaps of the H β C structure, the chemical shifts of the protons at the gaps changed, whereas the outer protons remained unaffected. The ^1H NMR spectra of AZE, H β C, and their inclusion complex are demonstrated in Figure 1c. The ^1H chemical shifts of AZE and H β C were in accordance with the previous studies [25,34]. The disappearance of the carboxylic acid peak at 11.96 of ^1H NMR spectrum of AZE confirmed the formation of the inclusion complex. (AZE ^1H NMR (300 MHz, DMSO- d_6) δ 11.96 (s, 2H), 3.4 (H $_2$ O in DMSO), 2.49 (DMSO), 2.17 (t, J = 5.4 Hz, 4H), 1.45 (p, J = 7.0 Hz, 4H), 1.29 – 1.17 (m, 6H). H β C ^1H NMR (300 MHz, DMSO- d_6) δ 5.88, 5.73, 5.01, 4.82, 4.55, 3.72, 3.60, 3.4 (H $_2$ O in DMSO), 2.49 (DMSO), 1.01. AZE-H β C inclusion complex ^1H NMR (300 MHz, DMSO- d_6) δ 5.87, 5.73, 5.00, 4.81, 4.51, 3.59, 3.4 (H $_2$ O in DMSO), 2.49 (DMSO), 2.18, 2.16, 2.14, 1.45, 1.22, 1.00.).

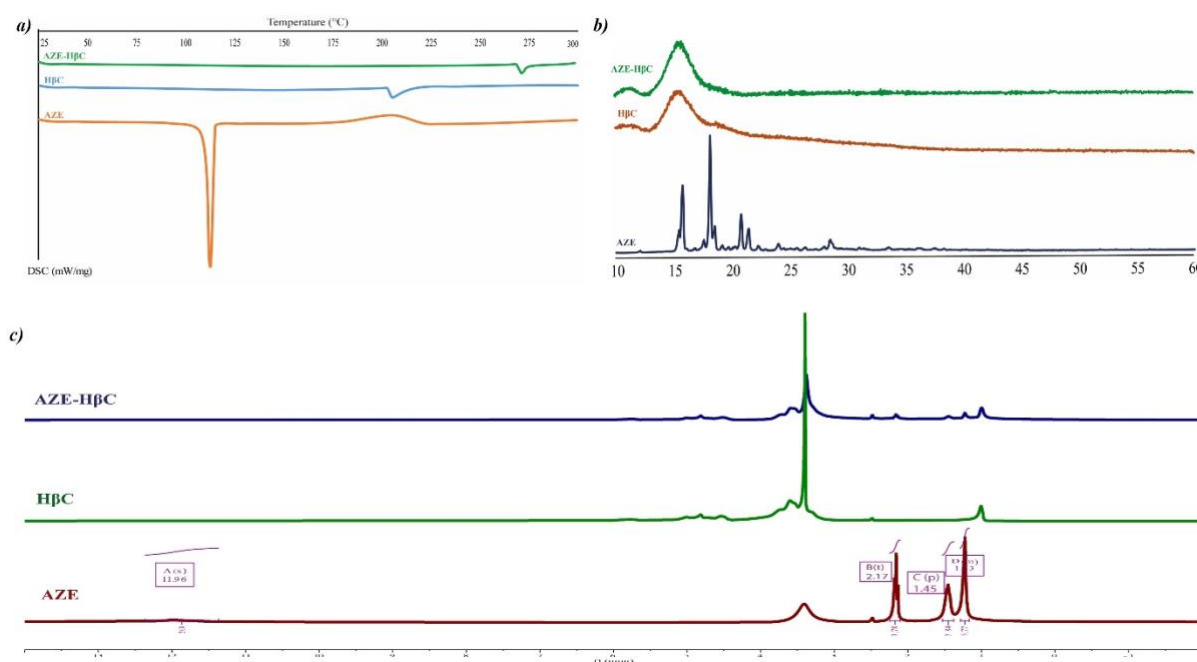


Figure 1. Characterization results of AZE- H β C inclusion complex (a) DSC, (b) XRD (c) NMR plots of AZE, H β C, and AZE-H β C inclusion complex.

2.3. Characterization of hydrogels

2.3.1. Viscosity, Flow Characteristics, and Spreadability

Viscosity, flow characteristics, and spreadability are parameters that affect the application of semi-solid formulations. These characterization results are presented in Figure 2. The viscosities of the gels were found as 214.40 ± 7.79 (F1) and 225.70 ± 9.42 Pa.s (F2). As seen in Figure 2a, while the viscosity of the gels increased ($p > 0.05$) with increasing HPMC, their spreadability decreased as expected ($p > 0.05$). This inverse relationship between viscosity and spreadability has been confirmed in the literature [35–37]. The very high viscosity of the gels resulted in low spreadability. The viscosity of blank formulations (AZE and H β C free) containing 2.5% of HPMC (G1_{free}) and 3% of HPMC (G2_{free}) was determined as 4.35 and 8.60 Pa.s in preliminary studies, respectively, which mean a very low viscosity compared to F1 and F2. AZE-H β C-based hydrogel, which was in a fluid form before the addition of HPMC, reached a much more viscous structure with the addition of HPMC. The coexistence of H β C and HPMC caused the gel to reach a very high viscosity. Also, the use of high amounts of CD increased the viscosity [38]. It was also shown that the viscosity of xanthan gum- or guar gum-based gels was improved even at low concentrations due to the formation of bridges between adjoint polymer chains presenting in a strong connection network [39].

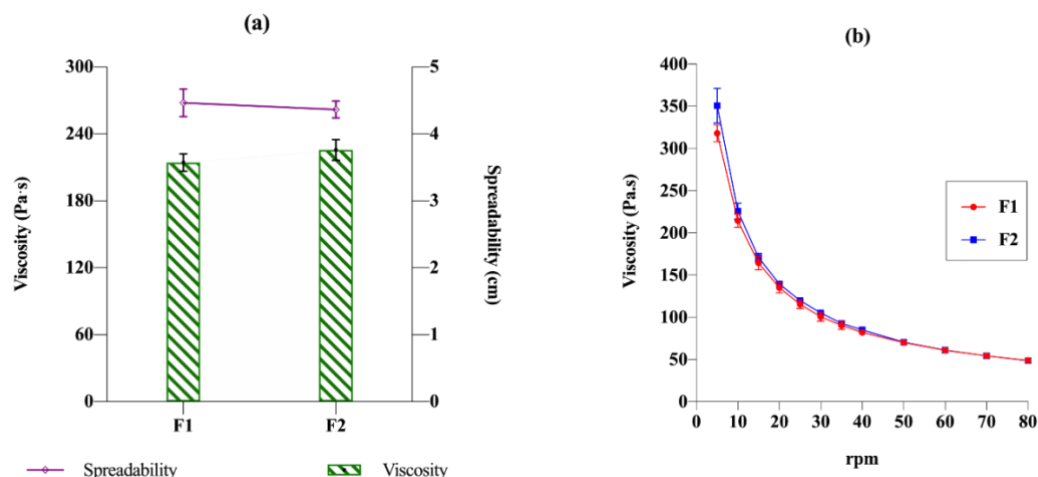


Figure 2. (a) Results of viscosity and spreadability of the gels (n=3). The viscosity of gels was measured at 10 rpm by Brookfield DV2T-RV viscometer. While the viscosity increased, the spreadability decreased. (b) F1 and F2 showed pseudo-plastic type of non-Newtonian flow characteristic by Brookfield DV2T-RV viscometer (n=3).

The rheological character of the gels was evaluated with the viscosity-rpm graph obtained by determining the viscosity of the gel at different rotation speeds (Figure 2b). As the spindle rotation speed increased, the viscosity of the gel decreased. While this decrease rate was very high between 5 and 40 rpm, it was lower between 40 and 80 rpm. The graph meant that the hydrogels showed pseudoplastic flow type. Vanti *et al.* investigated the change in viscosity of HPMC-based hydrogel formulations depending on the change in the rotation speed of the spindle. As the rotation speed increased, it was seen that the decreasing viscosity indicated the flow rate of the non-Newtonian flow and these systems had shear-thinning properties. At high rotation speeds (50-100 rpm), this decrease was less, and the measurements were repeated from 100 rpm to 2.5 rpm (down viscosity ramp), it was stated that the same results were reached and the viscosity did not change depending on the time [40]. Based on the increased shear stress, polymer particles moved in the direction of flow and reduced the viscosity of the system, that was, the pseudoplastic flow type was seen [41]. As a result, our findings were compatible with the literature.

2.3.2. Mechanical properties

Mechanical properties or texture profile analysis (TPA) was originally proposed by Jones *et al.* as a suitable method to characterize semisolid dosage forms. Emerging mechanical parameters such as hardness, adhesiveness, and compressibility affect the therapeutic outcomes of drug formulations [42,43]. The results of the mechanical properties of the formulations are given in Table 1. Hardness is the highest force value obtained from the first compression period of the probe and this value is an important parameter that affects the applicability of formulation [43,44]. Compressibility, which refers to the force required to deform the formulation during the first compression process, is presented as the area under the curve of the first cycle in the TPA graph [44,45]. F2 had higher values than F1 in both parameters ($p < 0.05$). While the adhesiveness affecting the residence time of the hydrogel formulations at the application site was found to be 0.287 ± 0.161 N.mm (F1) and 0.410 ± 0.195 N.mm (F2) ($p < 0.05$), the cohesiveness indicating the resistance of the internal structure of the hydrogel to break down were 0.871 ± 0.111 (F1) and 0.892 ± 0.117 (F2) ($p > 0.05$). As the polymer concentration increased, the number of solids dispersed increased, causing the formulation to be less coherent [46]. Elasticity, which presents the rate at which hydrogels return to their original state when the deformation force is removed, indicates the interaction between the polymers and the epithelial barrier [4,47]. The elasticity values of the formulations were quite close to each other ($p > 0.05$). Consequently, as the amount of HPMC used as a gel-forming agent increased, the hardness, compressibility, and adhesiveness increased but no significant change was observed in the elasticity and the cohesiveness. The increase in the hardness and compressibility of the gel with the increasing polymer concentration was parallel with the viscosity. In a study, it was shown that viscosity, hardness, and adhesiveness increased with the increase in polymer concentration of the gels prepared with chitosan (0.5-1%) [48]. In another study using polycarbophil or HPMC, they showed

that the hardness and compression of vaginal mucoadhesive gel formulations increased as their viscosities increased [49]. The findings obtained are compatible with the literature.

Table 1. Results of mechanical properties of AZE-H β C based hydrogels (n=3).

Formulation	Hardness (N \pm SD)	Compressibility (N.mm \pm SD)	Adhesiveness (N.mm \pm SD)	Cohesiveness (\pm SD)	Elasticity (\pm SD)
F1	0.151 \pm 0.033	0.942 \pm 0.201	0.287 \pm 0.161	0.871 \pm 0.111	0.963 \pm 0.001
F2	0.201 \pm 0.017	1.327 \pm 0.063	0.410 \pm 0.195	0.892 \pm 0.117	0.965 \pm 0.002

2.3.3. FTIR analysis

FTIR spectra of AZE, H β C, AZE-H β C inclusion complex, and HPMC are given in Figure 3, in addition to FTIR spectra of F1, F2, G1, G2 drug-loaded and blank formulations. The sharp peak detected at 1686 cm⁻¹ demonstrated the carboxylic acid groups located at the two ends of the molecule, whereas the peaks of the aliphatic carbon chain were observed around 2800-2950 cm⁻¹ [4, 50]. The peak visible at 3339 cm⁻¹ of the H β C spectrum showed -OH stretching vibration. The peaks at 2925 cm⁻¹, 1409 cm⁻¹, and 1024 cm⁻¹ belong to C-H stretching vibration, C-H bending vibration, and primary alcohol C-O stretching vibration, respectively [25]. The peak at 1050 cm⁻¹ of the HPMC spectrum represented C-O stretching vibration. The peak at 3417 cm⁻¹, on the other hand, demonstrated the out-of-plane bending vibration of the hydroxyl group [51]. The FTIR spectra of the formulations indicated the characteristic peaks of their components. Thus, H β C characteristic peaks could be observed in F1 and F2 formulations containing AZE inclusion complex, whereas they could not be detected in G1 and G2 formulations containing AZE. However, their intensities could change in a wide range depending on their proportions in the formulations.

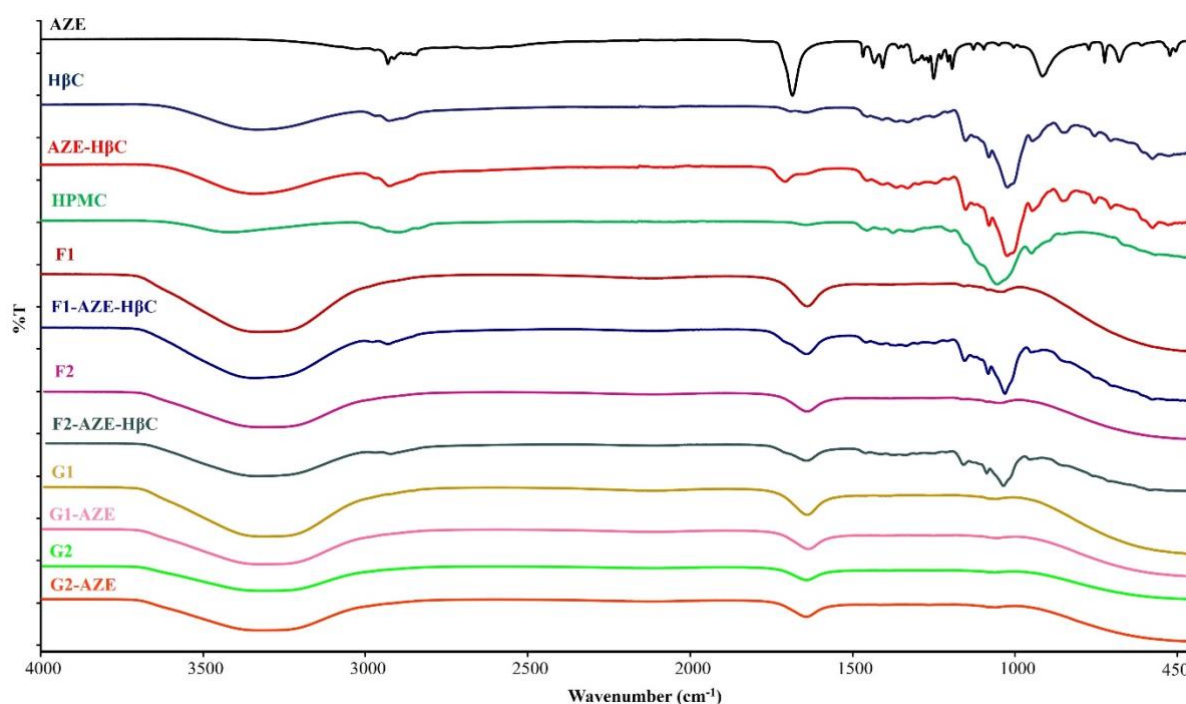


Figure 3. FTIR spectra of AZE, H β C, AZE-H β C complex, HPMC, AZE-loaded and blank formulations.

2.3.4. HPLC assay and content uniformity

For the quantification of AZE, the HPLC method was developed, and validation studies were carried out. The equation of the calibration graph obtained as a result of the studies was $y = 2.7505x - 2.7926$ and the correlation coefficient (r^2) was 0.9995. The retention time was found to be approximately 5 min. For validation studies, accuracy and recovery, precision (repeatability, reproducibility), and stability analyses were performed. All parameters obtained conformed to the ICH guideline [52]. The limit of detection (LOD) and limit of quantification (LOQ) values of AZE were found to be 0.49 \pm 0.13 μ g/mL and 1.50 \pm 0.39 μ g/mL,

respectively. The content uniformity of the AZE-H β C hydrogels was $104.21 \pm 1.80\%$ (F1) and $101.36 \pm 2.53\%$ (%). These results indicated that the formulations were homogeneous.

2.4. *Ex vivo* bioadhesion studies

Bioadhesion, defined as the ability of a system to adhere to biological tissue or mucosa, is directly related to the residence time and effectiveness of semi-solid systems in the application area [53,54]. CD and HPMC are polymers with bioadhesive characteristics. While the amount of H β C was constant, it was observed that the bioadhesion improved with increasing HPMC amount ($p < 0.05$, Figure 4). The result of F2 being more adhesive according to the *in vitro* TPA studies was confirmed by the results of the *ex vivo* bioadhesion study. Numerous studies have shown that bioadhesion improves as HPMC increases. Manna *et al.* [55] developed tenofovir-loaded vaginal gel formulations using HPMC (4-6%). As the HPMC ratio increased, they found that the viscosity of the gels and the bioadhesive capability enhanced. In a study using different types of HPMC (HPMC K4M, HPMC K10M, and HPMC K100M), the gel with the highest bioadhesive force was the formulation containing HPMC K100M [56]. As the molecular weight or amount of the polymer increases, the result of the improvement of the bioadhesive ability of the formulation is compatible with the data of our study.

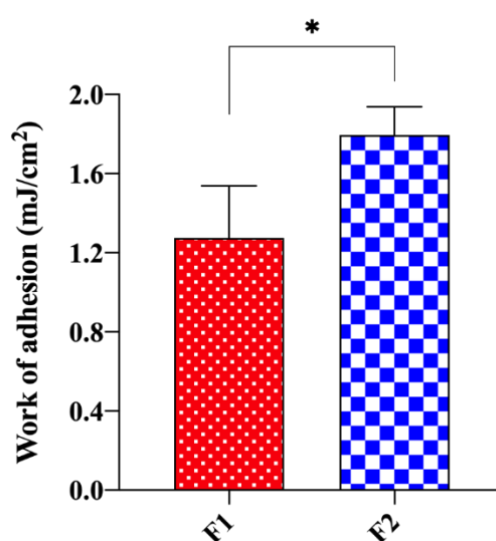


Figure 4. Results of *ex vivo* bioadhesion studies performed using a shaved Balb/c mice skin ($n=5$) (* $p < 0.05$).

2.5. Drug release studies

The results of drug release studies performed with the dialysis bag method are shown in Figure 5. Gel formulations containing dispersed AZE without H β C (G1 and G2) released 100% of the active ingredient within 8-10 h. In the formulations in which AZE was dissolved in a complex form with H β C (F1 and F2), cumulative AZE release reached 95% within 12 h. From the time of the first measurement, the drug release from the classical gels was higher than that of the H β C-based hydrogels (Figure 5a). In classical AZE gels, the drug was dispersed, not dissolved. As the temperature increases, the solubility of AZE increases [29,30], this situation increased the diffusion of dispersed AZE from the classic gels. At the same time, the classical gel formulations had a fluid structure and lower viscosity than H β C-based hydrogels. This also contributed to the faster release of G1 and G2 compared to F1 and F2. As the viscosity of semi-solid formulations increases, drug release occurs more slowly [57–59].

However, since there was no significant difference between the viscosity of F1 and F2 (Figure 2), no difference was observed in the release profiles either (Figure 5a). Conversely, AZE release from Azelderm cream was quite slower (at least $p < 0.05$) and, as seen in Figure 5b, AZE release from the cream was found to be $38.43 \pm 3.25\%$ after 12 h, which was quite low compared to all hydrogel formulations ($p < 0.0001$).

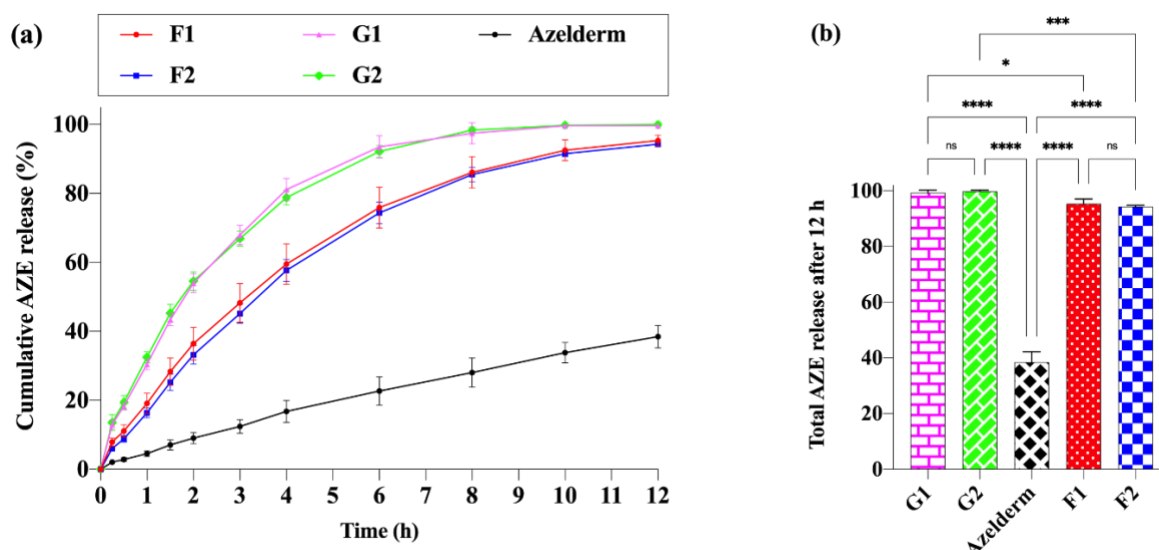


Figure 5. Results of *in vitro* drug release studies (n=5). (a) Drug release profiles of AZE-H β C based hydrogels (F1 and F2), classic hydrogels (G1 and G2), and Azelderm cream performed by dialysis bag method. (b) Graph represents total AZE release at the end of 12 h. p means statistical value: * p<0.05, *** p<0.001, **** p<0.0001.

The similarity between drug release profiles was investigated using the dissimilarity factor (f1) and similarity factor (f2), which are the model-independent kinetics. A f1<15 or f2>50 indicates that the drug release profiles are similar [60]. The obtained results are presented in Table 2. The f1 and f2 among the classic hydrogels (G1 and G2) were 2 and 88, respectively, while the f1 and f2 between the H β C-based hydrogels (F1 and F2) were found to be 5 and 80, respectively. Consequently, the change in HPMC concentration did not cause a difference in the AZE release profiles. However, no similarity was found between the release profiles of classic and H β C-based hydrogels. The presence of AZE dissolved or dispersed in the gel or the viscosities of the formulations caused a change in the release characteristics. The lower viscosity of G1 and G2 contributed to faster release. Also, although AZE was not dissolved in G1 and G2, the temperature of the release medium was 32°C. Therefore, the solubility of AZE increased due to the lukewarm temperature of the medium. This may have contributed to the faster release of AZE from G1 and G2 compared to F1 and F2. Since AZE is a white crystalline solid [61], G1 and G2 formulations were white due to dispersed AZE, while F1 and F2 were transparent due to dissolved AZE. However, it was observed that the white color of G1 and G2 started to become transparent rapidly after being immersed in the release medium and the gel in the dialysis bag was completely clear within a few hours. As a result, the rapid dissolution of dispersed AZE and lower viscosity of classic gels may have resulted in a faster release of classic gels compared to H β C-based hydrogels.

It was concluded that there was no similarity between the release profiles of hydrogel formulations developed with Azelderm with the highest f1 and lowest f2 values obtained. This result may be related to the fact that while Azelderm was oil-based, the gels were water-based. Patel *et al.* have developed psoralen-loaded cream and hydrogel formulations. They reported that the release of the drug from gel formulations was higher compared to the cream [62]. In another study, Sankar *et al.* reported that their hydrocortisone gel formulation reached a level of release about 2 times higher than the hydrocortisone commercial cream [63]. As a matter of fact, an active substance from formulation such as ointment and cream shows poor release due to an oily base of the formulation, which can slow the diffusion of the drug. On the contrary, gels allow the release of drugs to be easier and faster, as they are compatible with the dissolution medium. At the same time, the fact that the active substances with hydrophobic character have a higher affinity toward the oily base may also cause this result [64].

Table 2. Results of difference (f1) and similarity factors between formulation pairs.

	f1 (difference factor)	f2 (similarity factor)
F1-F2	5	80
F1-G1	29	42
F1-G2	29	42
F1-Azelderm	70	21
F2-G1	35	39
F2-G2	35	39
F2-Azelderm	69	22
G1-G2	2	88
G1-Azelderm	77	15
G2-Azelderm	77	15

Among the kinetic models applied to the release profiles, the highest r^2 was obtained with Higuchi kinetics (Table 3). Higuchi kinetics is the most suitable kinetic model to describe the release profile of semi-solids [65–67]. It appeared that drug release is both diffusion- and erosion-controlled. The fact that the n value, which is presented as the slope of the line in Korsmeyer-Peppas kinetics, was between 0.5 and 1, shows that the drug release was controlled by non-Fickian diffusion and Case II transport, hence the time-independent, diffusion and erosion control, and was therefore compatible with Higuchi kinetics [60].

Table 3. Results of kinetic models applied drug release data of AZE-H β C based hydrogels (F1 and F2).

Formulation	Zero order (r^2)	First order (r^2)	Higuchi (r^2)	Hixson-Crowell (r^2)	Korsmeyer-Peppas n (r^2)
F1	0.9182	0.7267	0.9859	0.8074	0.7570
F2	0.9224	0.7172	0.9853	0.8059	0.8463

3. CONCLUSION

Acne is a chronic skin disorder that seriously affects people of all ages. AZE, which is generally used as a topical cream and gel formulation in acne treatment, has poor water solubility. This may cause permeability problems of AZE. In this study, the AZE-H β C inclusion complex was prepared. It was found that 1.17 M of H β C was sufficient to dissolve 1 M of AZE. The AZE-H β C inclusion complex was confirmed in the results of characteristic studies (NMR, DSC, FTIR, and XRD analyses). Then, the viscosity of the formulations prepared hydrogel by the addition of HPMC was found between 214 and 225 Pa.s. It was seen that there was a positive relationship between the viscosity of hydrogels and hardness, compressibility, and adhesiveness. On the one hand, the developed formulations exhibited a similar release profile with the classic AZE hydrogels (H β C free), on the other hand, higher release results were obtained than the commercial cream formulation. Similar release rates between H β C free hydrogels and AZE-H β C hydrogels were associated with the lower viscosity of H β C free hydrogels and the increase in the solubility of the AZE due to the temperature of the dissolution medium. The findings obtained showed that the AZE-H β C based hydrogels developed for acne treatment will be an alternative formulation for the topical application of AZE.

4. MATERIALS AND METHODS

4.1. Materials

AZE was obtained from Acros Organics, USA. H β C was obtained from Shandong Binzhou Zhiyuan Biotechnology, China. Potassium dihydrogen phosphate was obtained from Merck, USA. Ortho-phosphoric acid was obtained from Isolab, Germany. Phosphate buffer tablet (pH 7.4) was obtained from Sigma, USA. Hydroxypropyl methylcellulose K4M (medium molecular weight) was kindly gifted from Colorcon, Germany.

4.2. Preparation of AZE-H β C complex

In order to enhance the water solubility of AZE, inclusion complexes with H β C were obtained by preparing their aqueous solutions according to the previous studies [68]. For this purpose, the amount of CD that can dissolve AZE (1 M) was investigated by adding different molar concentrations of H β C to 1 mL of distilled water in a vial. Firstly, H β C was added to the vial containing distilled water and stirred on a magnetic

stirrer (MR-Hei Standard, Heidolph, Germany) at 300 rpm until dissolved. Subsequently, AZE (1 M) was added to the solution and stirred at the same speed for 3 h. The solubility of AZE was observed visually, and then the amount of dissolved AZE was determined by an HPLC device. Samples were prepared using 11 different molar ratios of H β C from 0.17 to 2 M to dissolve 1 M of AZE (Table 4). All studies were carried out at ambient temperature. For HPLC analysis, the samples were individually transferred to microcentrifuge tubes and centrifuged at 14,000 rpm for 30 min (3-18KS, Sigma, Germany) at room temperature. Then, the supernatant was taken and diluted with a mobile phase used for HPLC analysis and analyzed at 208 nm.

Table 4. Molar ratio of AZE-H β C complex.

Sample	AZE (M)	H β C (M)
C1	1	0.17
C2	1	0.33
C3	1	0.50
C4	1	0.67
C5	1	0.84
C6	1	1.00
C7	1	1.17
C8	1	1.34
C9	1	1.50
C10	1	1.67
C11	1	2.00

4.3. Characterization of AZE-H β C inclusion complex

4.3.1. DSC analysis

AZE, H β C, and the inclusion complex of AZE-H β C measurements were carried out using Netzsch DSC 200 F3 differential scanning calorimeter (Germany). The temperature increase was applied with a rate of 25 °C/min in 25–300 °C range. During the experiments, approximately 2 mg of the sample was put on an aluminum pan. The analysis was performed under a nitrogen atmosphere with 20 mL/min speed. An empty pan was used as the reference standard.

4.3.2. XRD analysis

X-ray powder diffraction analysis was carried out to investigate the host-guest interaction and decipher the structure of the formulations. AZE, H β C, and the inclusion complex of AZE-H β C measurements were performed using an X-ray Diffractometer (Rigaku DMax 2200 XRD, Rigaku Corp., Tokyo, Japan) system with Cu-K α radiation and 10° to 90° (2 θ) sequential collection. The step time was taken 0.5 s. The duration of the acquisition was 1 h with 0.5 s step time.

4.3.3. ¹H NMR analysis

¹H-NMR spectra of AZE, H β C, and the inclusion complex of AZE-H β C were obtained using VARIAN Infinity Plus 300 MHz (Varian Company, USA). DMSO-d₆ was used for the analyte solutions.

4.4. Formulation of AZE-H β C hydrogels

The lowest molar ratio of H β C, which provided water solubility of AZE, was selected for the preparation of hydrogel. Before proceeding to the formulation studies, the sample considered suitable was lyophilized, and it was visually checked whether it dissolved without leaving any particulate residue. To prepare the hydrogel, H β C was dissolved in a beaker containing distilled water. Then, AZE was added to the solution and stirred until dissolved. Finally, two different formulations were prepared by adding HPMC (2.5% and 3%, w/w) to the solutions (Table 5). The preparation of the hydrogels was carried out at ambient temperature and using the magnetic stirrer.

Table 5. Composition of AZE-H β C based hydrogels (w/w).

Formulation	AZE (M)	H β C (M)	HPMC (% w/w)	Distilled water (up to, % w/w)
F1	1	1.17	2.5	100
F2	1	1.17	3	100

4.5. Characterization of hydrogels

4.5.1. Viscosity and Flow Characteristics

The viscosity of hydrogels was determined at ambient temperature with a rotational viscometer (DV2T-RV, Brookfield, USA). For this, a probe (RV7) was dipped into the gel after enough amount of the hydrogel was transferred into a metal tube of the device. The viscosity and rheological character of the hydrogel were determined by studying at different rotation speeds (10-80 rpm). The studies were performed in triplicate.

4.5.2. Spreadability

To determine the spreadability of the gel, two glass plates (20x20 cm²) were used. One g of gel was weighed in the center of the glass plate and the same sized plate was covered onto the first plate [44]. The study was repeated three times for each batch.

4.5.3. Mechanical properties

A texture analyzer (TA.XTplusC, Stable Micro Systems, Haslemere, Surrey, UK) equipped with a 5 kg load cell was used to characterize the mechanical properties of the gel. A required amount of gel (25 g) was transferred to a plastic container. The test, pre-test, and post-test speed were set to 2 mm/s. A probe (P10) was descended into the gel with a constant speed to a depth of 10 mm and left for 10 seconds. It then moved upward at the same speed toward the surface of the gel. The mechanical properties (adhesiveness, compressibility, hardness, elasticity, and cohesiveness) of the gel were evaluated using the software of the device (Exponent Connect 8.0.7.0) on the graph obtained as a result of repeating this process twice [4]. The process was performed in triplicate for each formulation.

4.5.4. FTIR analysis

Fourier-transform infrared spectra of AZE, H β C, AZE-H β C, and HPMC, as well as the AZE-loaded and blank formulations of F1, F2, G1, and G2 formulations were obtained using a Perkin Elmer Spectrum Two with ATR equipment (USA) with 4 cm⁻¹ resolution and in the spectral range of 4000-400 cm⁻¹.

4.5.5. HPLC assay and content uniformity

A quantitative determination method of AZE was developed using acetonitrile and pH 4 phosphate buffer (v/v, 25:75) as a mobile phase and C18 column (C18, 150*4.6 mm, 5 μ m, InertSustain, GL Sciences, Japan) by an HPLC device (Agilent 1100, USA) equipped a UV detector. HPLC method parameters were as follows: 25 °C of a column temperature, 1.2 mL of a flow rate, 100 μ L of an injection volume, and 208 nm of a wavelength [4]. While determining linearity with 10 different samples of AZE in the concentration range of 20-400 μ g/mL, the method was validated in accordance with the ICH guideline [52]. Before starting the analysis, the device was conditioned with the mobile phase for at least 40 min.

In order to determine the content uniformity of the gels, 1 g of gel was taken from different points of the gel and dispersed in 200 mL of pH 7.4 phosphate buffer for 1 h. After the samples were filtered using a membrane filter (0.2 μ m, cellulose acetate, Isolab, Germany), they were analyzed by HPLC. Three replicates were run for each batch.

4.6. Ex vivo bioadhesion studies

The bioadhesive character of the gel was determined by the texture analyzer. The shaved Balb/c mice skin was fixed to the probe with a rubber band. One g of gel was filled into a glass beaker. The probe was moved downwards, and the gel was contacted with the skin with 1 N of applied force for 120 s. At the end of the time, the probe moved upwards and separated from the gel [60,65]. While the highest force obtained when leaving the gel surface was recorded as the bioadhesive force, the distance at the detachment time and the bioadhesive force provided the work of adhesion to be obtained using the device software and the following Equation 1.

$$\text{Work of adhesion (mJ cm}^{-2}\text{)} = \text{AUC}_{1-2} / \pi r^2 \text{ (Equation 1)}$$

πr^2 : The area of the probe/skin surface

AUC_{1-2} : The area under the curve in the force-distance graph

4.7. Drug release studies

Drug release studies were carried out with a dialysis bag method according to previous studies with minor changes [60,69,70]. As the control group, both AZE-loaded HPMC hydrogels (H β C free) and Azelderm cream (Orva, Türkiye) were used. To prepare H β C-free HPMC hydrogels (classic gels), HPMC was first dissolved in water, then AZE was added and mixed until a homogeneous appearance was achieved. These gels are named G1 (2.5% of HPMC) and G2 (3% of HPMC). After all formulations were weighed into the dialysis bag to contain an equal amount of AZE, the mouth of the bags was closed with magnetic clips. The dialysis bag was immersed in a beaker containing 200 mL of pH 7.4 phosphate buffer. The study was performed at 32 \pm 1°C and 50 rpm. Aliquots of 1 mL were withdrawn at the specified times from 30 min up to 12 h. After each sampling, the mediums were made up with the fresh buffer. The samples were filtered through a membrane filter (0.2 μ m, cellulose acetate, Isolab, Germany) and analyzed by HPLC. The study was carried out in five repetitions.

Release kinetics (zero order, first order, Higuchi, Hixson-Crowell, Korsmeyer-Peppas) were applied to elucidate the release character of the formulations. In addition, difference factor (f1) and similarity factor (f2) as model-independent kinetics were investigated in order to assess whether there was a similarity between the release profiles.

4.8. Statistical analyses

Statistical analyses were performed using GraphPad Prism Software (Version 9.0.1., CA, USA). To evaluate statistically significant differences, one-way analysis of variance (ANOVA) was used between three or more groups. Besides, Tukey's multiple comparisons test was done. $p < 0.05$ was accepted as statistically significant. All values are presented as the mean \pm SD (standard deviation).

Acknowledgements: The authors thank Colorcon (Germany) for kindly gifting HPMC. The authors thanks to Prof.Dr. Ali Osman Aydın, Assoc.Prof. Emrah Bulut, and Assoc.Prof. Zafer Şahin for contributing characterization studies of AZE-H β C

Author contributions: Concept – M.D.A.; Design – M.D.A.; Supervision – M.D.A.; Resources – M.D.A., Ü.C.E.; Materials – M.D.A., Ü.C.E.; Data Collection and/or Processing – M.D.A., N.D., M.G., E.S.K., Ü.C.E.; Analysis and/or Interpretation – M.D.A., Ü.C.E.; Literature Search – M.D.A., N.D., Ü.C.E.; Writing – M.D.A., N.D., Ü.C.E.; Critical Reviews – M.D.A., Ü.C.E.

Conflict of interest statement: The authors declared no conflict of interest.

REFERENCES

- [1] Tan AU, Schlosser BJ, Paller AS. A review of diagnosis and treatment of acne in adult female patients. *Int J Womens Dermatol.* 2018; 4: 56-71. <https://doi.org/10.1016/j.jjwd.2017.10.006>
- [2] Moradi Tuchayi S, Makrantonaki E, Ganceviciene R, Dessinioti C, Feldman SR, Zouboulis CC. Acne vulgaris. *Nat Rev Dis Prim.* 2015; 1: 15029. <https://doi.org/10.1038/nrdp.2015.29>
- [3] Prasad SB. Acne vulgaris: A review on pathophysiology and treatment. *Asian J Pharm Clin Res.* 2016; 9: 54-59.
- [4] Arpa MD, Seçen İM, Erim ÜC, Hoş A, Üstündağ Okur N. Azelaic acid loaded chitosan and HPMC based hydrogels for treatment of acne: Formulation, characterization, in vitro-ex vivo evaluation. *Pharm Dev Technol.* 2022; 27: 268-281. <https://doi.org/10.1080/10837450.2022.2038620>
- [5] Özer S, Şenel B, Yazan Y. Preparation and in vitro evaluation of in situ gelling system containing lithium carbonate for parenteral administration. *Polym Bull.* 2020; 77: 599-622. <https://doi.org/10.1007/s00289-019-02764-5>
- [6] Jung H, Kim MK, Lee JY, Choi SW, Kim J. Adhesive hydrogel patch with enhanced strength and adhesiveness to skin for transdermal drug delivery. *Adv Funct Mater.* 2020; 30: 1-10. <https://doi.org/10.1002/adfm.202004407>
- [7] Üstündağ Okur N, Çağlar EŞ, Arpa MD, Karasulu HY. Preparation and evaluation of novel microemulsion-based hydrogels for dermal delivery of benzocaine. *Pharm Dev Technol.* 2017; 22: 500-510. <https://doi.org/10.3109/10837450.2015.1131716>
- [8] Pourtalebi Jahromi L, Rothhammer M, Fuhrmann G. Polysaccharide hydrogel platforms as suitable carriers of liposomes and extracellular vesicles for dermal applications. *Adv Drug Deliv Rev.* 2023; 200: 115028. <https://doi.org/10.1016/j.addr.2023.115028>
- [9] Mahmood A, Sharif A, Muhammad F, Sarfraz RM, Abrar MA, Qaisar MN, Anwer N, Amjad MW, Zaman M. Development and in vitro evaluation of (β -cyclodextrin-g-methacrylic acid)/na⁺-montmorillonite nanocomposite hydrogels for controlled delivery of lovastatin. *Int J Nanomedicine.* 2019; 14: 5397-5413. <http://doi.org/10.2147/IJN.S209662>
- [10] Wangsawangrung N, Choipang C, Chairwut S, Ekabutr P, Suwantong O, Chuysinuan P, Techasakul S, Supaphol P. Quercetin/hydroxypropyl- β -cyclodextrin inclusion complex-loaded hydrogels for accelerated wound healing. *Gels.*

- 2022;8(9):573. <https://doi.org/10.3390/gels8090573>
- [11] Yurttaş G, Demirel M, Genç L. Inclusion complexes of fluconazole with β -cyclodextrin: Physicochemical characterization and in vitro evaluation of its formulation. *J Incl Phenom Macrocycl Chem*. 2011; 70: 429-435. <https://doi.org/10.1007/s10847-010-9908-z>
 - [12] Davis ME, Brewster ME. Cyclodextrin-based pharmaceuticals: Past, present and future. *Nat Rev Drug Discov*. 2004; 3: 1023-1035. <https://doi.org/10.1038/nrd1576>
 - [13] Bilensoy E, Rouf MA, Vural I, Şen M, Hincal AA. Mucoadhesive, thermosensitive, prolonged-release vaginal gel for clotrimazole: β -cyclodextrin complex. *AAPS PharmSciTech*. 2006; 7(2): E54. <https://doi.org/10.1208/pt070238>
 - [14] Poulson BG, Alsulami QA, Sharfalddin A, El Agammy EF, Mouffouk F, Emwas A-H, Jaremko L, Jaremko M. Cyclodextrins: Structural, chemical, and physical properties, and applications. *Polysaccharides*. 2021; 3: 1-31. <https://doi.org/10.3390/polysaccharides3010001>
 - [15] Sherje AP, Kulkarni V, Murahari M, Nayak UY, Bhat P, Suvarna V, Dravyakar B. Inclusion complexation of etodolac with hydroxypropyl-beta-cyclodextrin and auxiliary agents: Formulation characterization and molecular modeling studies. *Mol Pharm*. 2017; 14: 1231-1242. <https://doi.org/10.1021/acs.molpharmaceut.6b01115>
 - [16] Pazoki-Toroudi H, Nilforoushzadeh MA, Ajami M, Jaffary F, Aboutaleb N, Nassiri-Kashani M, Firooz A. Combination of azelaic acid 5% and clindamycin 2% for the treatment of acne vulgaris. *Cutan Ocul Toxicol*. 2011; 30:286-291. <https://doi.org/10.3109/15569527.2011.581257>
 - [17] Webster G. Combination azelaic acid therapy for acne vulgaris. *J Am Acad Dermatol*. 2000; 43: 47-50. <https://doi.org/10.1067/mjd.2000.108318>
 - [18] Kainz JT, Berghammer G, Auer-Grumbach P, Lackner V, Perl-Convaletius S, Popa R, Wolfesberger B. Azelaic acid 20 % cream: effects on quality of life and disease severity in adult female acne patients. *J Dtsch Dermatol Ges*. 2016;14(12):1249-1259. <https://doi.org/10.1111/ddg.12889>
 - [19] Hung WH, Chen PK, Fang CW, Lin YC, Wu PC. Preparation and evaluation of azelaic acid topical microemulsion formulation: In vitro and in vivo study. *Pharmaceutics*. 2021; 13(3): 410. <https://doi.org/10.3390/pharmaceutics13030410>
 - [20] Kiti K, Suwantong O. The potential use of curcumin- β -cyclodextrin inclusion complex/chitosan-loaded cellulose sponges for the treatment of chronic wound. *Int J Biol Macromol*. 2020; 164: 3250-3258. <https://doi.org/10.1016/j.ijbiomac.2020.08.190>
 - [21] Argenziano M, Haimhoffer A, Bastiancich C, Jicsinszky L, Caldera F, Trotta F, Scutera S, Alotto D, Fumagalli M, Musso T, Castagnoli C, Cavalli R. In vitro enhanced skin permeation and retention of imiquimod loaded in β -cyclodextrin nanosponge hydrogel. *Pharmaceutics*. 2019;11(3):138. <https://doi.org/10.3390/pharmaceutics11030138>
 - [22] Kaur N, Puri R, Jain SK. Drug-cyclodextrin-vesicles dual carrier approach for skin targeting of anti-acne agent. *AAPS PharmSciTech*. 2010; 11: 528-537. <https://doi.org/10.1208/s12249-010-9411-2>
 - [23] Ascenso A, Vultos F, Ferrinho D, Salgado A, Filho SG, Ferrari V, Simoes S, Marques HC. Effect of tretinoin inclusion in dimethyl-beta-cyclodextrins on release rate from a hydrogel formulation. *J Incl Phenom Macrocycl Chem*. 2012; 73: 459-465. <https://doi.org/10.1007/s10847-011-0002-y>
 - [24] Manosroi J, Apriyani MG, Foe K, Manosroi A. Enhancement of the release of azelaic acid through the synthetic membranes by inclusion complex formation with hydroxypropyl- β -cyclodextrin. *Int J Pharm*. 2005; 293: 235-240. <https://doi.org/10.1016/j.ijpharm.2005.01.009>
 - [25] Kumar A, Rao R. Enhancing efficacy and safety of azelaic acid via encapsulation in cyclodextrin nanosponges: development, characterization and evaluation. *Polym Bull*. 2020; 78: 5275-5302. <https://doi.org/10.1007/s00289-020-03366-2>
 - [26] Hassan DS, Hasary HJ. The impact of viscosity on the dissolution of naproxen immediate-release tablets. *J Taibah Univ Med Sci*. 2023; 18: 687-695. <https://doi.org/10.1016/j.jtumed.2022.12.009>
 - [27] Permanadewi I, Kumoro AC, Wardhani DH, Aryanti N. Effect of viscosity on iron encapsulation using alginate as a carrying agent in a controlled spray drying process. *Food Res*. 2022; 6: 56-67. [https://doi.org/10.26656/fr.2017.6\(5\).613](https://doi.org/10.26656/fr.2017.6(5).613)
 - [28] Wang Z, Xiang H, Dong P, Zhang T, Lu C, Jin T, Chai KY. Pegylated azelaic acid: Synthesis, tyrosinase inhibitory activity, antibacterial activity and cytotoxic studies. *J Mol Struct*. 2021; 1224: 129234. <https://doi.org/10.1016/j.molstruc.2020.129234>
 - [29] Breathnach A. Azelaic acid: A new agent in the treatment of acne: history, metabolism and biochemistry. *J Dermatolog Treat*. 1989; 1: 7-10. <https://doi.org/10.3109/09546638909094474>
 - [30] Zimmermann F, Meux E, Mieloszynski JL, Lecuire JM, Oget N. Ruthenium catalysed oxidation without CCl₄ of oleic acid, other monoenic fatty acids and alkenes. *Tetrahedron Lett*. 2005; 46: 3201-3203. <https://doi.org/10.1016/j.tetlet.2005.03.052>
 - [31] Cid-Samamed A, Rakmai J, Mejuto JC, Simal-Gandara J, Astray G. Cyclodextrins inclusion complex: Preparation methods, analytical techniques and food industry applications. *Food Chem*. 2022; 384: 132467. <https://doi.org/10.1016/j.foodchem.2022.132467>
 - [32] Cevher E, Şensoy D, Zloh M, Mülazımoğlu L. Preparation and characterisation of natamycin: γ -cyclodextrin inclusion complex and its evaluation in vaginal mucoadhesive formulations. *J Pharm Sci*. 2008; 97: 4319-4335. <https://doi.org/10.1002/jps.21312>
 - [33] Jordheim LP, Degobert G, Diab R, Peyrottes S, Périgaud C, Dumontet C, Fessi H. Inclusion complexes of a nucleotide

- analogue with hydroxypropyl-beta-cyclodextrin. *J Incl Phenom Macrocycl Chem.* 2009; 63: 11-16. <https://doi.org/10.1007/s10847-008-9483-8>
- [34] Olteanu AA, Aramă C-C, Radu C, Mihăescu C, Monciu C-M. Effect of β -cyclodextrins based nanosponges on the solubility of lipophilic pharmacological active substances (repaglinide). *J Incl Phenom Macrocycl Chem.* 2014; 80: 17-24. <https://doi.org/10.1007/s10847-014-0406-6>
- [35] Deuschle VCKN, Norbert Deuschle RA, Bortoluzzi MR, Athayde ML. Physical chemistry evaluation of stability, spreadability, in vitro antioxidant, and photo-protective capacities of topical formulations containing *Calendula officinalis* L. leaf extract. *Brazilian J Pharm Sci.* 2015; 51: 63-75. <https://doi.org/10.1590/S1984-82502015000100007>
- [36] Afreen U, Fahelelbom KM, Shah SNH, Ashames A, Almas U, Khan SA, Yameen MA, Nisar N, Bin Asad MHH, Murtaza G. Formulation and evaluation of niosomes-based chlorpheniramine gel for the treatment of mild to moderate skin allergy. *J Exp Nanosci.* 2022; 17: 467-495. <https://doi.org/10.1080/17458080.2022.2094915>
- [37] Dixit K, Mohapatra D, Senapati PC, Panda R, Sahu AN. Formulation development and evaluation of *Lawsonia inermis* extract loaded hydrogel for wound dressing application. *Indian J Pharm Sci.* 2022; 84.
- [38] Thurein SM, Lertsuphotvanit N, Phaechamud T. Physicochemical properties of β -cyclodextrin solutions and precipitates prepared from injectable vehicles. *Asian J Pharm Sci.* 2018; 13: 438-449. <https://doi.org/10.1016/j.ajps.2018.02.002>
- [39] Rao GCS, Ramadevi K, Sirisha K. Effect of β -cyclodextrin on rheological properties of some viscosity modifiers. *Indian J Pharm Sci.* 2014; 76: 545-548.
- [40] Vanti G, Wang M, Bergonzi MC, Zhidong L, Bilia AR. Hydroxypropyl methylcellulose hydrogel of berberine chloride-loaded escinosomes: Dermal absorption and biocompatibility. *Int J Biol Macromol.* 2020; 164: 232-241. <https://doi.org/10.1016/j.ijbiomac.2020.07.129>
- [41] Szulc-Musiół B, Siemiradzka W, Dolińska B. Formulation and evaluation of hydrogels based on sodium alginate and cellulose derivatives with quercetin for topical application. *Appl Sci.* 2023; 13. <https://doi.org/10.3390/app13137826>
- [42] Jones DS, Woolfson AD, Djokic J. Texture profile analysis of bioadhesive polymeric semisolids: Mechanical characterization and investigation of interactions between formulation components. *J Appl Polym Sci.* 1996; 61: 2229-2234. [https://doi.org/10.1002/\(SICI\)1097-4628\(19960919\)61:12<2229::AID-APP24>3.0.CO;2-0](https://doi.org/10.1002/(SICI)1097-4628(19960919)61:12<2229::AID-APP24>3.0.CO;2-0)
- [43] Hurler J, Engesland A, Poorahmary Kermay B, Škalco-Basnet N. Improved texture analysis for hydrogel characterization: Gel cohesiveness, adhesiveness, and hardness. *J Appl Polym Sci.* 2012; 125: 180-188. <https://doi.org/10.1002/app.35414>
- [44] Arpa MD, Yoltaş A, Onay Tarlan E, Şenyüz ÇŞ, Sipahi H, Aydın A, Üstündağ Okur N. New therapeutic system based on hydrogels for vaginal candidiasis management: formulation-characterization and in vitro evaluation based on vaginal irritation and direct contact test. *Pharm Dev Technol.* 2020; 25: 1238-1248. <https://doi.org/10.1080/10837450.2020.1809457>
- [45] Fonseca-Santos B, dos Santos AM, Rodero CF, Daflon Gremião MP, Chorilli M. Design, characterization, and biological evaluation of curcumin-loaded surfactant-based systems for topical drug delivery. *Int J Nanomedicine.* 2016; 11: 4553-4562. <https://doi.org/10.2147/IJN.S108675>
- [46] Basu S, Maity S. Preparation and characterisation of mucoadhesive nasal gel of venlafaxine hydrochloride for treatment of anxiety disorders. *Indian J Pharm Sci.* 2012; 74: 428-433.
- [47] Capanema NS V, Mansur AAP, de Jesus AC, Carvalho SM, de Oliveira LC, Mansur HS. Superabsorbent crosslinked carboxymethyl cellulose-PEG hydrogels for potential wound dressing applications. *Int J Biol Macromol.* 2018; 106: 1218-1234. <https://doi.org/10.1016/j.ijbiomac.2017.08.124>
- [48] Emani S, Vangala A, Buonocore F, Yarandi N, Calabrese G. Chitosan hydrogels cross-linked with trimesic acid for the delivery of 5-fluorouracil in cancer therapy. *Pharmaceutics.* 2023; 15(4): 1084. <https://doi.org/10.3390/pharmaceutics15041084>
- [49] Limpongsa E, Tabboon P, Tuntiyasawasdikul S, Sripanidkulchai B, Pongjanyakul T, Jaipakdee N. Formulation and in vitro evaluation of mucoadhesive sustained release gels of phytoestrogen diarylheptanoids from *Curcuma comosa* for vaginal delivery. *Pharmaceutics.* 2023; 15(1): 264. <https://doi.org/10.3390/pharmaceutics15010264>
- [50] Radwan-Pragłowska J, Janus Ł, Piątkowski M, Sierakowska A, Matysek D. ZnO nanorods functionalized with chitosan hydrogels crosslinked with azelaic acid for transdermal drug delivery. *Colloids Surf B Biointerfaces.* 2020; 194: 111170. <https://doi.org/10.1016/j.colsurfb.2020.111170>
- [51] Arpa MD, Ünükür MZ, Erim ÜC. Formulation, characterization and in vitro release studies of terbinafine hydrochloride loaded buccal films. *J Res Pharm.* 2021; 25: 667-680. <https://doi.org/10.29228/jrp.58>
- [52] ICH. ICH Harmonised Tripartite Guideline (2005) Validation of Analytical Procedures: Text and Methodology Q2(R1). International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, Geneva, 1-13. 2005. <https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf> (accessed on 24 October 2023).
- [53] Kumar K, Dhawan N, Sharma H, Vaidya S, Vaidya B. Bioadhesive polymers: Novel tool for drug delivery. *Artif Cells, Nanomedicine Biotechnol.* 2014; 42: 274-283. <https://doi.org/10.3109/21691401.2013.815194>
- [54] Amorós-Galicia L, Nardi-Ricart A, Verdugo-González C, Arroyo-García CM, García-Montoya E, Pérez-Lozano P, Suñé-Negre JM, Suñé-Pou M. Development of a standardized method for measuring bioadhesion and mucoadhesion that is applicable to various pharmaceutical dosage forms. *Pharmaceutics.* 2022; 14(10): 1995. <https://doi.org/10.3390/pharmaceutics14101995>

- [55] Manna S, Lakshmi US, Racharla M, Sinha P, Kanthal LK, Kumar SPN. Bioadhesive HPMC gel containing gelatin nanoparticles for intravaginal delivery of tenofovir. *J Appl Pharm Sci.* 2016; 6: 22-29. <https://doi.org/10.7324/JAPS.2016.60804>
- [56] Cho CW, Choi JS, Shin SC. Enhanced local anesthetic action of mepivacaine from the bioadhesive gels. *Pak J Pharm Sci.* 2011; 24: 87-93.
- [57] Tas C, Ozkan Y, Okyar A, Savaser A. In vitro and ex vivo permeation studies of etodolac from hydrophilic gels and effect of terpenes as enhancers. *Drug Deliv.* 2007; 14: 453-459. <https://doi.org/10.1080/10717540701603746>
- [58] Shamma RN, Salah Ad-din I, Abdeltawab NF. Dapsone- gel as a novel platform for acne treatment: In vitro evaluation and in vivo performance and histopathological studies in acne infected mice. *J Drug Deliv Sci Technol.* 2019; 54: 101238. <https://doi.org/10.1016/j.jddst.2019.101238>
- [59] Tuğcu-Demiröz F, Acartürk F, Erdoğan D. Development of long-acting bioadhesive vaginal gels of oxybutynin: Formulation, in vitro and in vivo evaluations. *Int J Pharm.* 2013; 457: 25-39. <http://dx.doi.org/10.1016/j.ijpharm.2013.09.003>
- [60] Arpa MD, Kesmen EE, Biletekin SN. Novel sprayable thermosensitive benzydamine hydrogels for topical application : Development , characterization , and in vitro biological activities. *AAPS Pharmscitech.* 2023; 1-16. <https://doi.org/10.1208/s12249-023-02674-w>
- [61] Gollnick H, Layton A. Azelaic acid 15% gel in the treatment of rosacea. *Expert Opin Pharmacother.* 2008; 9: 2699-2706. <https://doi.org/10.1517/14656566.9.15.2699>
- [62] Patel NA, Patel NJ, Patel RP. Comparative development and evaluation of topical gel and cream formulations of psoralen. *Drug Discov Ther.* 2009; 3: 234-242. <http://www.ncbi.nlm.nih.gov/pubmed/22495634>
- [63] Sankar V, Praveen C, Prasanth KG, Srinivas CR, Ruckmann K. Formulation and evaluation of a proniosome hydrocortisone gel in comparison with a commercial cream. *Pharmazie.* 2009; 64: 731-734. <https://doi.org/10.1691/ph.2009.9095>
- [64] Maru S, Gathu LW, Mathenge AW, Okaru AO, Kamau F, Chepkwony HK. In Vitro drug release studies of metronidazole topical formulations through cellulose membrane. *East Cent African J Pharm Sci.* 2012; 15: 57-62.
- [65] Çağlar EŞ, Karaotmarlı Güven G, Üstündağ Okur N. Preparation and characterization of carbopol based hydrogels containing dexpanthenol. *Ankara Univ Eczac Fak Derg.* 2023; 47. <https://doi.org/10.33483/jfpau.1195397>
- [66] Erol İ, 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. <https://doi.org/10.1080/10837450.2020.1765180>
- [67] Xu X, Al-Ghabeish M, Krishnaiah YSR, Rahman Z, Khan MA. Kinetics of drug release from ointments: Role of transient-boundary layer. *Int J Pharm.* 2015; 494: 31-39. <http://doi.org/10.1016/j.ijpharm.2015.07.077>
- [68] de Castro DO, Tabary N, Martel B, Gandini A, Belgacem N, Bras J. Controlled release of carvacrol and curcumin: bio-based food packaging by synergism action of TEMPO-oxidized cellulose nanocrystals and cyclodextrin. *Cellulose.* 2018; 25: 1249-1263. <https://doi.org/10.1007/s10570-017-1646-6>
- [69] Küçüktürkmen B, Öz UC, Bozkir A. Diklofenak sodyum yüklü polimerik nanopartiküllerin intra-artiküler uygulaması için in situ hidrojel formülasyonu. *Turkish J Pharm Sci.* 2017; 14: 56-64. <https://doi.org/10.4274/tjps.84803>
- [70] Nounou MM, El-Khordagui LK, Khalafallah NA, Khalil SA. In vitro release of hydrophilic and hydrophobic drugs from liposomal dispersions and gels. *Acta Pharm.* 2006; 56: 311-324.