J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg., 47(3): 1041-1052, 2023
Doi: 10.33483/jfpau.1319262

ORIGINAL ARTICLE / ÖZGÜN MAKALE

PREPARATION AND *IN VITRO* CHARACTERIZATION OF LIDOCAINE LOADED *ALOE VERA* GEL FORMULATION FOR THE TREATMENT OF BURN WOUNDS

YANIK TEDAVİSİNDE KULLANILMAK ÜZERE LİDOKAİN İÇEREN ALOE VERA JEL FORMÜLASYONUNUN HAZIRLANMASI VE İN VİTRO KARAKTERİZASYONU

Umay Merve GÜVEN[1], Tilbe ÇEVİKELLİ[1]*, Sanem SONGÜLOĞLU[1], Serpil DEMİRCİ KAYIRAN[2]

[1] Cukurova University, Faculty of Pharmacy, Department of Pharmaceutical Technology, 01330, Adana, Turkey

[2] Cukurova University, Faculty of Pharmacy, Department of Pharmaceutical Botany, 01330, Adana, Turkey

ABSTRACT

Objective: *In this study, topically applied in situ gel formulations were aimed to design for the modulation of burns, with the active ingredient lidocaine and the gel gained from the Aloe vera plant. The prepared in situ gels were in the liquid form at the room temperature and gelled at the body temperature and adhered to the wound surface, resulting in higher drug residence time. By improving the characteristic properties of the in situ gels, it is aimed to improve patient compliance by obtaining higher local lidocaine concentration.*

Material and Method: *In situ gel formulations separated by giving different gel codes were examined with characteristic analyses. Within the scope of these examinations, measurement of gelation temperature, pH measurement, in vitro lidocaine release, viscosity and rheological properties and the texture profile of the formulations were determined.*

Result and Discussion: *Poloxamer 407 based in situ gels designed for topical treatment containing Aloe vera gel and lidocaine have been shown to increase skin residence time. Among the formulations prepared with different content ratios of the polymers Poloxamer 407, Poloxamer 188, HPMC and CMC, the gels coded F_5 and A_{21} showed acceptable gelation temperature for topical use and sustained lidocaine release for 24 hours. According to these findings, it can be revealed that Poloxamer 407-HPMC based in situ gel formulation may be an effective alternative for topical burn*

* **Corresponding Author / Sorumlu Yazar:** Tilbe Çevikelli
e-mail / e-posta: tcevikelli@cu.edu.tr, **Tel. / Phone:** +903223387334

Submitted / Gönderilme : 23.06.2023
Accepted / Kabul : 05.09.2023
Published / Yayınlanma : 20.09.2023

treatment.
Keywords: *Aloe vera, burn treatment, in situ gel, lidocaine, topical formulation*

ÖZ

Amaç: *Bu çalışma kapsamında Aloe vera bitkisinden elde edilen jelin, lidokain etken maddesi ile birlikte yanık tedavisinde topikal olarak uygulanacak in situ jel formülasyonlarının geliştirilmesi amaçlanmıştır. Formülasyonların karakteristik özellikleri iyileştirilerek, oda sıcaklığında sıvı formda olan in situ jellerin vücut sıcaklığında jelleşerek yara yüzeyine yapışma suretiyle daha yüksek lokal ilaç konsantrasyonu elde edilerek hasta uyuncunun artırılması hedeflenmiştir.*
Gereç ve Yöntem: *Farklı jel kodları verilerek ayrılan in situ jel formülasyonlarının karakteristik özellikleri analiz edilmiştir. Bu incelemeler kapsamında jelleşme sıcaklığının tespiti, pH ölçümü, in vitro lidokain salım çalışması gerçekleştirilmiş ve in situ jellerin viskozite ve reolojik özellikleri ile tekstür profil analizleri değerlendirilmiştir.*
Sonuç ve Tartışma: *Aloe vera jeli ve lidokain içeren, topikal tedavi için tasarlanmış Poloxamer 407 bazlı in situ jellerin deri üzerinde kalış süresini uzattığı ve lokal lidokain konsantrasyonunu artırabileceği belirlenmiştir. Poloxamer 407, Poloxamer 188, HPMC ve CMC'nin farklı oranları ile hazırlanan in situ jel formülasyonları arasında F_5 ve A_{21} kodlu jeller topikal kullanım için kabul edilebilir jelleşme sıcaklığı göstermiş ve 24 saat lidokain salımı gerçekleştirmiş olup, Aloe vera ve lidokain içeren Poloxamer 407-HPMC bazlı in situ jel formülasyonunun topikal yanık tedavisinde etkili bir alternatif olabileceği sonucuna varılmıştır.*
Anahtar Kelimeler: *Aloe vera, in situ jel, lidokain, topikal formülasyon, yanık tedavisi*

INTRODUCTION

Burn is a significant injury trauma that may result from heat, freezing, electricity, chemicals or radioactive factors and can lead to life-threatening injuries depending on the extent and depth of the damage [1,2]. Burn injuries are the fourth most common trauma sources around the world [3].

In treatment of burn wounds, application of systemic or topical agents are well studied. Main cause of the usage of topical antimicrobial agents is to diminish the development of burn wound sepsis and its related morbidity and mortality [4]. In addition to clinical treatment, medicinal plants take a significant role in the healing of burn wounds due to the various content of alkaloids, flavonoids, terpenoids, tannins, saponins and phenolic compounds [5].

From the literature, it is well established that phytochemicals in medicinal plants show positive effect in the healing process of burn wounds with different burn degrees. This positive effect may be resulted from the antimicrobial, anti-inflammatory, antioxidant, astringent, collagen synthesis stimulator and perfusion enhancing properties of the medicinal plants.

Aloe vera is from the Asphodelaceae family, which is native to Africa, Madagascar and the Arabian Peninsula and cultivated in the South-west coasts of Turkey [5,6]. It is a every green perennial, shrubby plant with rosette leaves. The leaves are thick and fleshy, have grey to green color range, with some variations showing white stains on their upper and lower stem surfaces. The margin of the leaf is milled and has small white teeth. The flowers have yellow color and leaves' color is in green grey spectrum [7,8].

Aloe vera contains high amount of water (99-99.5 %), and solid content (0.5-1 %) is composed of minerals, vitamins, enzymes, polysaccharides, organic acids and phenolic compounds which are soluble in the water or oil [8]. Various parts of the *Aloe vera* contains approximately 70 nutrients as well as 200 active compositions including amino acids, saponins, anthraquinones, lignin, salicylic acid [9]. Anti-inflammatory, laxative, antihistaminic, fibroblast proliferative, burns and wound healing properties of topical *Aloe vera* application in gel form have also been reported [10,11]. It is also used in treatment of skin traumas, as well as, frostbite, rashes, cold sores, dry skin, skin ulcers, psoriasis and seborrheic eczema [6,12,13].

Aloe vera gel is frequently used in the topical treatment of minor burns, sunburns and X-ray burns [6]. The anti-inflammatory property of the *Aloe vera* contributes to the improvement of the inflammatory process caused by the burn injury. Since blocking the formation of vasoactive prostanoids prevents vasoconstriction thrombosis and the progressive ischemic necrosis known to ocur in thermal and electric

burns as a result of thromboxane production, the application of *Aloe vera* can prevent the progressive nature of thermal injury and the provides control of the bacterial growth in the burn wound [14].

Local anesthetics has activity on the sodium ion channels to decrease the permeability of cell membranes; by preventing depolarization and the conduction of electric impulses [15]. Lidocaine belongs to the amide class local anesthetics, and preferred in inhibiting sense of pain with nerve blockade, as stabilizing the neuronal membrane by blocking the ionic fluxes of initiation and transmission of impulses [16,17].

Topical application of an agent refers to a method in which the formulation is applied to superficial regions; such as the skin or ocular, otic and vaginal tissues for the treatment of local diseases [18]. Despite the diversity of formulation systems, semi-solid formulations are frequently used in topical applications [9]. Topical administration provides a great advantage as avoiding the risks associated with intravenous therapy [19,20]. Gels are semi-solid formulations prepared with a suitable gelling agent, possesses the viscosity varying between 1000 and 100000 mPa.s. Gels provide higher solubilization of drugs due to its higher water content in comparison to the creams and ointments. Additionally, gels can hydrate skin and facilitate the drug transport by retaining considerable amount of transepidermal water [21].

In situ gelling formulations are polymeric carriers that are in solution form before contacting to the body, but transform into the gel construction at the physiological conditions [22-24]. The transition from solution to gel phase is dependent on one or more of different stimuli; such as pH shift, temperature menagement, solvent change, ultraviolet radiation, and the content of particular ions or molecules. Thermogels transform from solution to gel with temperature modulation; while they are in liquid form at the room temperature (20-25°C), they turn into gel form when they contact to the body fluids (32-37°C) [25]. *In situ* gel formulations have gained great interest in the last few years as they provide an advantage over conventional delivery systems to achieve plasma drug concentration [22,26]. A rising number of *in situ* gel forming systems have been studied and many patents have been reported for their application in a variety of biomedical fields, including drug delivery [26].

MATERIAL AND METHOD

Chemicals

Lidocaine (Sigma Aldrich, Germany) as an active agent, Poloxamer 407® (P-407) (Sigma-Aldrich, Germany), Poloxamer 188® (P-188) (Sigma-Aldrich, Germany), hydroxypropyl methylcellulose (HPMC) (Sigma-Aldrich, Germany), carboxymethyl cellulose (CMC) (Sigma-Aldrich, Germany) as the polymer for *in situ* gelling systems. Benzalkonium chloride (Sigma Aldrich, Germany) is preferred as the preservative and distilled water is preferred as solvent.

Plant Material

Aloe vera samples were obtained from Çukurova University Ali Nihat Gökyiğit Medicinal and Aromatic Plants Garden, in January 2022. Collected *Aloe vera* samples were separated from leaves and homogenized in the laboratory to obtain *Aloe vera* gel.

Preparation of Gel Formulations

In situ gel formulations were prepared by the cold method with different polymer concentrations [27,28]. Shortly, weighed amount of Poloxamer 407 (12%-22% w/v) was dissolved in the distilled water and stirred on a magnetic stirrer to obtain a clear solution at least 12 hours at 4°C. Lidocaine concentration (5% w/v) was kept constant for all the formulations and added to the each solution with continuous mixing. HPMC and CMC solutions was prepared separately by incorporating a given amount (0.5% - 5% w/v) in water and then, mixed the with Poloxamer 407 solution. As following, different amount of *Aloe vera* gel (10-20-25-20% w/v) was added to prepared *in situ* gel formulations.

Determination of Sol-Gel Transition Temperatures

The temperature at the phase transition from sol to gel phase is recorded as the sol-gel transition temperature. The gelation temperatures were decided by the tube rotation method [29].

The gel sample was taken into a glass vial and temperature was gradually increased by the water bath. The specific temperature which the sample turn into gel from the sol gel form was recorded. The sol-gel transition temperature studies were done in triplicate for each formulation.

Determination of Lidocaine

Analytic validation by UV for Lidocaine was performed with phosphate buffer (pH 6.8) at the wavelength of 263 nm [13,30]. Partial validation was evaluated in the scope of linearity, precision and accuracy parameters [31]. The standard curves (n=3) were studied at the 50, 100, 150, 200, 250, 300, 400, 500 and 1000 µg/ml of concentrations. Concentrations of 100, 200, 400 µg/ml (n=3) were studied for precision while 50, 300, 500 µg/ml (n=3) were studied for accuracy.

Organoleptic Evaluation and pH Analysis

The color, odor and state of prepared *in situ* gels were evaluated by physical appearance for organoleptic determination. pH values of the *in situ* gels were determined with digital pH meter (WTW Profi Lab. pH 597, Germany) in triplicate and average values with deviations were recorded.

Examination of Rheological Behavior

The dynamic properties of the gels were measured using the Haake Rheometer I (Thermo Fisher Scientific Inc., Essen, Germany) (n=3). The rheologic characteristics of *in situ* gels were measured at 25 ± 0.5°C and 37± 0.5°C. The sample was places on the platform and shear rate evaluation was done between 0-2000 s^{-1}. RheoWin 4.87.0006 (Haake®) software was used to evaluate the results [32].

Texture Profile Analysis (TPA) of Gel Formulations

Mechanical characteristics of the gels including cohesiveness, adhesiveness and hardness were analyzed using Texture Analyzer (TA.XT. Plus C, Stable Micro System, Haslemere, Surry, UK). 10 mm diameter Perspex probe (SNSP/10, h: 10 mm) was used to measurement with 5 kg loading capacity. *In situ* gels were measured by placing 10 g of gels into a 25 mm beaker at 37± 0.5°C. Test parameters are given: Speed Before Test 2 mm/s, Test Speed 2mm/s Speed After Test 2 mm/s, Trigger Force 0.001 N.

***In vitro* Release Study**

In vitro drug release studies were conducted using dialysis membrane (Sigma-Aldrich, Germany; Molecular weight cut-off = 14,000 Da) using phosphate buffer as dissolution medium [33]. 1 ml of *in situ* gel formulation was placed in dialysis bags in 40 ml of dissolution medium of phosphate buffer with 100 rpm rate of stirring. At determined time intervals, 1 ml of samples were collected and were analyzed by UV spectrophotometer. Phosphate buffer was replaced by the same amount of media to remain sink conditions. Experiments were conducted in triplicate.

RESULT AND DISCUSSION

In this study, for the preparation of thermosensitive *in situ* gelling system of Lidocaine and *Aloe vera,* the cold method was used. *Aloe vera* was preferred due to anti-inflammatory, wound and burn healing properties of topical *Aloe vera* application in gel form have been reported, beside its moisturizing and soothing effects [10,11]. *Aloe vera* gel was gained from the leaves of the *Aloe vera* plant. *Aloe vera* gel was obtained by slicing the two leaves of the plant from the base (Figure 1) [34].

All the formulations were visually evaluated in light against alternative black and white backgrounds before and after gelling. Most of *in situ* gels prepared for this study were transparent at all test temperatures (25 and 37°C). The formulations prepared were found to be visually homogenous and clear, with no phase separation.

Sol-gel transition temperature was determined by visual inspection for different concentrations of gel. The measurements of sol-gel transition temperature were conducted by the tube rotation method (n=3). As predicted, it was established that gelation temperature decreased with the increasing content of Poloxamer 407. Some formulations tested did not exhibit gelling properties at any temperature (in the range of 20°C to 50°C). With the aim of ensure gelation of the thermoreversible gel at body

physiological temperature, a gelation temperature 37°C was selected. Compositions and sol-gel transition temperature of the selected *in situ* gel formulations prepared are detailed in Table I. Characterization studies were carried out with formulations F_5 and A_{21}.

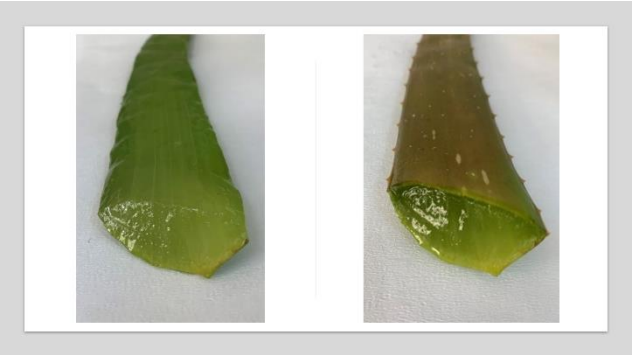

Figure 1. Leaves of the *Aloe vera* plant

Table 1. Composition percentage of *in situ* gel formulations and sol-gel transition temperatures

Code	P407 (%)	HPMC (%)	*Aloe vera* gel (%)	Lidocaine (%)	Benzalkonium chloride (%)	Sol-Gel Temp. (±0.5°C, n=3)
F_1	18	0.5	-	5	0.01	30.5°C
F_2	18	1	-	5	0.01	32.8°C
F_3	18	2.5	-	5	0.01	Solid Below 5°C
F_4	18	5	-	5	0.01	Solid Below 5°C
F_5	20		-	5	0.01	35.3°C
F_6	20	0.5	-	5	0.01	28.8°C
F_7	20	1	-	5	0.01	28.3°C
F_8	20	2.5	-	5	0.01	Solid Below 5°C
F_9	20	5	-	5	0.01	Solid Below 5°C
F_{10}	22	0.5	-	5	0.01	25.5°C
F_{11}	22	1	-	5	0.01	25.6°C
F_{12}	22	2.5	-	5	0.01	Solid Below 5°C
F_{13}	22	5	-	5	0.01	Solid Below 5°C
A_1	18	0.5	10	5	0.01	31.8°C
A_2	18	0.5	20	5	0.01	Liquid Above 50°C
A_3	18	0.5	25	5	0.01	Liquid Above 50°C
A_4	18	1	10	5	0.01	Liquid Above 50°C
A_5	18	1	20	5	0.01	Liquid Above 50°C
A_6	18	2.5	10	5	0.01	Solid Below 5°C
A_7	18	2.5	20	5	0.01	Solid Below 5°C

Table 1 (*continue*). Composition percentage of *in situ* gel formulations and sol-gel transition temperatures

Code	P407 (%)	HPMC (%)	*Aloe vera* gel (%)	Lidocaine (%)	Benzalkonium chloride (%)	Sol-Gel Temp. (±0.5°C, n=3)
A_8	18	2.5	50	5	0.01	Solid Below 5°C
A_9	18	5	10	5	0.01	Solid Below 5°C
A_{10}	18	5	20	5	0.01	Solid Below 5°C
A_{11}	18	5	50	5	0.01	Solid Below 5°C
A_{12}	20	0.5	10	5	0.01	Liquid Above 50°C
A_{13}	20	0.5	20	5	0.01	Liquid Above 50°C
A_{14}	20	0.5	25	5	0.01	Liquid Above 50°C
A_{15}	20	1	10	5	0.01	31.8°C
A_{16}	20	1	15	5	0.01	Liquid Above 50°C
A_{17}	20	1	20	5	0.01	Liquid Above 50°C
A_{18}	20	1	25	5	0.01	Liquid Above 50°C
A_{19}	20	5	50	5	0.01	Solid Below 5°C
A_{20}	22	0.5	10	5	0.01	28.5°C
A_{21}	22	0.5	20	5	0.01	36.5°C
A_{22}	22	0.5	25	5	0.01	Liquid Above 50°C
A_{23}	22	1	10	5	0.01	27.5°C
A_{24}	22	1	20	5	0.01	31.8°C
A_{25}	22	1	22.5	5	0.01	33.3°C
A_{26}	22	1	25	5	0.01	Liquid Above 50°C
A_{27}	22	5	10	5	0.01	Solid Below 5°C
A_{28}	22	5	20	5	0.01	Solid Below 5°C
A_{29}	22	5	50	5	0.01	Solid Below 5°C

The quantification analyses of the polymeric gel formulations were conducted using a UV spectrophotometer. For partial validation of the analytical method for the determination of lidocaine content; linearity, accuracy, precision and selectivity properties were evaluated [31].

To obtain the calibration curve 50, 100, 150, 200, 250, 300, 400 and 500 µg/ml concentrations were studied. The equation and the curve of the Lidocaine concentration/Absorbance values were obtained.

The precision of the method was evaluated by recovery studies done in three concentration levels of 100, 200, 400 µg/ml. Results were calculated as 0.111±0.018; 0.278±0.019 and 0.584±0.014 in order. The analyses were conducted on the same day to evaluate repeatability or intra-day variability and on different days to determine the intermediate precision or inter-day variability. Samples were prepared in three concentration levels of 50, 300, 500 µg/ml The results were calculated with the equation

obtained from calibration curve and compared to the known concentrations, and the mean (%) recovery of samples were found to be 95.929%.

pH values of F_5 and A_{21} formulations prepared with Lidocaine were measured and mean and standard deviation values were calculated. The pH values of F_5 and A_{21} formulations were determined 5.864±0.020 and 5.567±0.032, respectively.

Rheological evaluation was performed to determine the flow properties of the formulation. The rheograms of the F_5 and A_{21} formulations containing lidocaine are presented at Figure 2. Measurements were done at both 25 and 37°C.

Mechanical properties of the *in situ* gels including cohesiveness, adhesiveness and hardness were determined and the results are presented at the Figure 3.

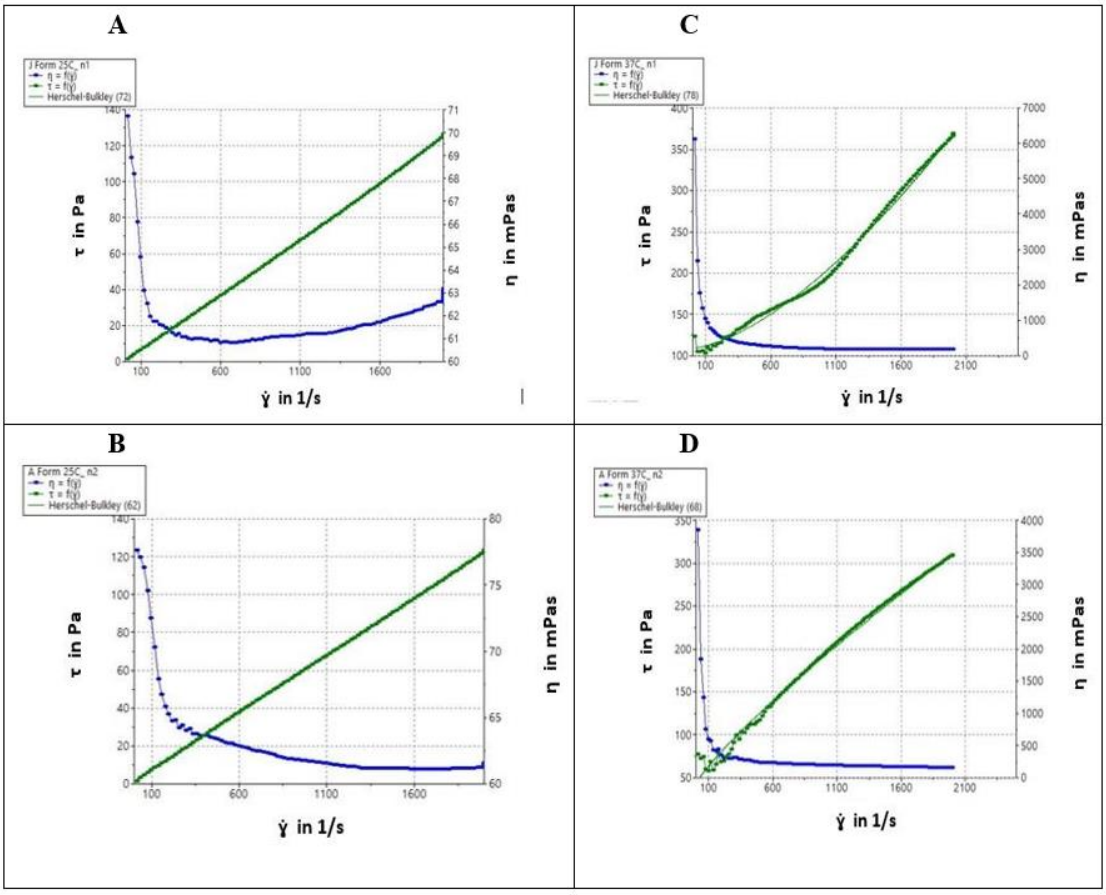

Figure 2. Rheograms of the F_5 and A_{21} formulations measured at 25°C and 37°C. (**A**: F_5 at 25°C, **B**: A_{21} at 25°C, **C**: F_5 at 37°C, **D**: A_{21} at 37°C). (τ: shear stress, γ̇: shear strain, Pa: stress in pascals; x axis = γ̇ in 1/s, y axis = τ in Pa)

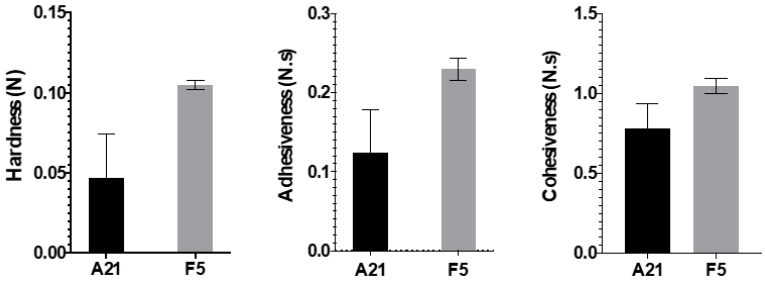

Figure 3. Texture profile analysis of the A_{21} and F_5 formulations (Mean ± SD; n = 3)

The cumulative drug release (%) of lidocaine from the *in situ* gel and lidocaine solution were calculated from the calibration curve of lidocaine. As shown in Figure 4, lidocaine solution has reached to 97.61 ± 2.14% just after 1.5 hours depending on solubility in the medium. For the formulation F_5 and A_{21} percentages of cumulative release at 24 hours were 73.39 ± 3.54%, 84.54 ± 2.66%, respectively.

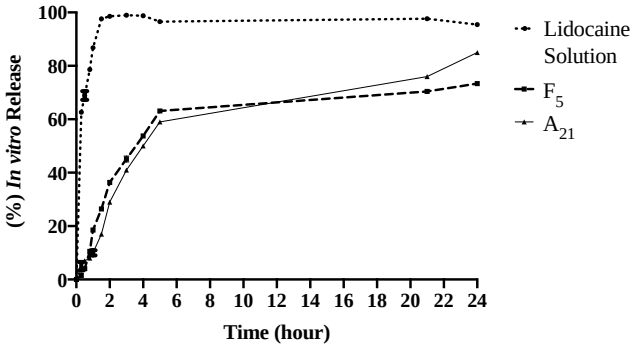

Figure 4. *In vitro* release profiles of lidocaine solution, F_5 and A_{21} *in situ* gel formulations (Mean ± SD; n = 3)

In this study, a well known anesthetic molecule, lidocaine, which is used in clinic for years; was combined with the *Aloe vera* plant due to its known activity in burn lesions, in order to improve therapeutic efficiency. Novel topical delivery of lidocaine and *Aloe vera* plant was designed as an *in situ* gelling system for modulation of burn wounds.

A significant reason why *in situ* gel systems gain great interest as a local application system in dermal diseases is that they can hold on to the injured skin surface for a long time, release the active substance continuously, and provide higher local drug concentration than conventional semi-solid dosage forms [35,36]. *In situ* gel systems provide ease of application on injured surfaces due to their flow properties at the solution form at room temperature [37,38]. In this study, P-407 and P-188 were used as temperature dependent gelling agents, and HPMC and CMC were preferred as conventional gelling agents. Poloxamer *in situ* gels are low viscous solutions at 25°C but turn into gels at physiological temperature. This property makes them optimum polymers for topical applications. In addition, their biodegradability, low toxicity and high stability advantages makes P-407 and P-118 potential polymers that can be used in temperature sensitive gelation systems [39,40]. HPMC is a polymer that is widely used in oral and topical pharmaceutical formulations and varies according to viscosity, molecular weight and degree of substitution. Due to its high water absorption capacity and gelling properties, HPMC has become an important material for drug delivery systems. It is chemically inert, compatible with packaging components, physically stable under physiological conditions and easily available [41]. CMC is preferred due to its high water solubility, high biocompatibility and low price, and is widely used in many areas [42].

In this study, the gel obtained from the *Aloe vera* plant was used regarding to its anti-inflammatory, moisturizing, soothing and wound healing effects. The effect of *Aloe vera* on sunburns, X-ray burns, thermal and electrical burns have been proven in various literature [43].

In addition to the therapeutic effect of *Aloe vera,* lidocaine, a local anesthetic belonging amide class, has been added to prevent sense of pain. It is aimed to develop a topical *in situ* gelling formulation using the anesthetic effect of lidocaine and the wound healing properties of the *Aloe vera* [14,15].

In situ gel formulations were prepared as a topical drug delivery system and gelation temperatures were evaluated to determine appropriate polymer ratios. The transition temperatures from solution to gel state depending on the polymer concentrations were evaluated. Since topical use was aimed in our study, the rates of gelation at body temperature were accepted as ideal [44,45]. Gelation temperatures above 25°C and below 37°C have been considered suitable. For gelation temperatures below the 25°C, a gel might easily be formed at room temperature; obstructing the preparation, handling and administration of the formulation. On the contrary, at gelation temperatures above the 37°C, a liquid

state may maintain after topical application [43,44,46]. It was found that the gelling temperature increased with the addition of *Aloe vera* in the formulations. For this reason, the formulations to be added *Aloe vera* were selected from gels with a gelling temperature below 36°C or formulations being in solid state below 5°C (Table 1). Some of the formulations remained in the solid state below 5°C (F_3, F_4, F_8, F_9, F_{12}, F_{13}, A_6, A_7, A_8, A_9, A_{10}, A_{11}, A_{19}, A_{27}, A_{28}, A_{29}) while some formulations remained in the liquid state above 50°C (A_2, A_3, A_4, A_5, A_{12}, A_{13}, A_{14}, A_{16}, A_{17}, A_{18}, A_{22}, A_{26}); thus eliminated from the further characterization studies. Remaining formulations (F_1, F_2, F_5, F_6, F_7, F_{10}, F_{11}, A_1, A_{15}, A_{20}, A_{21}, A_{23}, A_{24}, A_{25}) had sol-gel transition temperatures between 25-37°C. Characterization tests were carried out on F_5 and A_{21} coded gels due to their ideal gelling temperatures, 35.3 and 36.5°C respectively; providing appropriate sol-gel transition properties for physical application.

For partial validation of the analytical method for the quantification of lidocaine content; linearity, accuracy, precision and selectivity properties were evaluated [29].

As result of the linearity, the r^2 value of the standard curve is close to one, indicating the reliability of the obtained data. The linearity of the method was determined in the range of 50-500 μg/ml, and showed perfect correlation within the concentration range.

The (%) relative standard deviation values of the data obtained from the accuracy assay were found to be less than 2%. It has been shown that the method determined for quantification gives accurate results. The precision study was carried out in term of repeatability. Since the (%) relative standard deviations of the resulting data are less than 2%, the reproducibility of the study has been proven. None of the placebo formulations interfered at the wavelength of 263 nm, where lidocaine showed maximum absorbance. This data shows that the quantification method is specific to lidocaine and provides the required selectivity.

Skin pH is approximately in the range of 4-6 [47]. Despite the strong buffering capacity of the skin, the pH of topical formulations should be between 5.0 and 7.0 for safe application. When formulations with acidic pH value are applied, the patient may experience discomfort and skin irritation, while microbial growth may develop at the alkaline pH. The pH values of the formulations we prepared were found to be appropriate [48].

Evaluation of the rheological characteristics of *in situ* gels is one of the most significant parameters to predict their *in vivo* behavior. Dynamic viscosity (η′) is defined as the flow resistance of the formulation against oscillating motion. A higher dynamic viscosity value refers to higher resistance to flow [49]. In this study, it was observed that the viscosity of the gels in solution form was low at room temperature, while the viscosity of the gels increased at 37°C. In rheological measurements, Newtonian flow model is observed in shear stress versus shear velocity measurement at room temperature, while Non-Newtonian flow model is observed in measurements made at 37°C (Figure 2). Viscosity and rheology results support that the formulation behaviors differ depending on the temperature.

It has been shown that parameters of hardness, adhesion and cohesion are related to the ease of removal of the topical formulations from the packaging in which they are placed; the convenience of application to the surface on which they are applied, and the retention of the product in place. Therefore, texture profile analysis is frequently applied to identify formulations that may be appropriate for clinical application [45]. In this study, the hardness, adhesion and cohesion parameters of the texture profile analysis were evaluated. Hardness is defined as the force required for a predetermined deformation; with this parameter the degree of deformation of the sample is measured [47]. A low gel stiffness is desirable for providing the gel to be easily removed from the container and spread over the skin. In our study, the hardness values were found to be ideal, and it was determined that the hardness of the formulation containing *Aloe vera* was lower than the formulations which *Aloe vera* was not added (Figure 3). The adhesion parameter is established as the work required to overcome the attractive forces between the surface of the sample and the probe [51]. This parameter is related to the adhesive characteristics of the formulation; higher adhesion value provides more adhesion on the tissue surface, and this improves the desired retention time of the drug [49]. Cohesion is defined as the internal structural strength that maintains strong interconnections with a certain level of resistance to rupture during application [50]. It is defined as the structural deformation and strength of the internal bonds in the sample after shear stress [51,52]. Adhesion and cohesion values are high in F_5 gel and lower in A_{21} gel. HPMC and poloxamer polymers show mucoadhesive properties. However, although the polymer concentration is

higher in the F_5 formulation, the lower adhesion and cohesion values are thought to be related to *Aloe vera* gel content (Figure 3).

Diffusion through a dialysis membrane is a conventional technique to evaluate the drug release from colloidal dispersions and topical formulations [30]. *In vitro* release study shows that poloxamer based thermoresponsive *in situ* gel could significantly decrease the drug release compared to lidocaine solution (Figure 4). At the same time, the lidocaine and *Aloe vera* loaded *in situ* gel might display a higher burn wounds therapy effect compared with the conventional lidocaine solution due to quick and constant drug release profile.

In conclusion, lidocaine and *Aloe vera* containing *in situ* gel formulations were developed and characterized for treatment of burn wounds. Different content ratios of Poloxamer 407, Poloxamer 188, HPMC and CMC were used as gelling agents, and suitable gelation temperature for topical use was examined. F_5 and A_{21} coded formulations showed appropriate sol-gel transition temperature and characterized with further studies including pH, rheological properties, texture profile analysis and drug release profiles. Poloxamer 407 based *in situ* gels showed increased skin residence time, and provided *in vitro* lidocaine release for 24 hours. According to these results lidocaine and *Aloe vera* containing Poloxamer 407-HPMC based *in situ* gel formulations can be concluded as an effective alternative for topical treatment of burn wounds.

AUTHOR CONTRIBUTIONS

Concept: U.M.G., T.Ç., S.D.K.; Design: U.M.G., T.Ç., S.D.K.; Control: U.M.G.; Sources: U.M.G., S.D.K.; Materials: U.M.G., S.D.K.; Data Collection and Processing: U.M.G., T.Ç., S.S.; Analysis: U.M.G., T.Ç., S.S.; Literature Review: U.M.G., S.S.; Manuscript Writing: U.M.G., T.Ç.; Critical Review: U.M.G., T.Ç., S.D.K.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

REFERENCES

1. Evers, L.H., Bhavsar, D., Mailänder, P. (2010). The biology of burn injury. Experimental Dermatology, 19(9), 777-783. [CrossRef]
2. Şakrak, T., Köse, A.A., Karabağlı, Y., Çetin, C. (2011). Yanık ünitemizde yatarak tedavi gören hastalara ait 10 yıllık tarama sonuçlarımız. Türk Plastik Rekonstrüktif ve Estetik Cerrahi Dergisi, 18(3), 111-115.
3. Greenhalgh, D.G. (2019). Management of burns. New England Journal of Medicine, 380(24), 2349-2359. [CrossRef]
4. Atiyeh, B.S., Gunn, S.W., Hayek, S.N. (2005). State of the art in burn treatment. World Journal of Surgery, 29(2), 131-148. [CrossRef]
5. Göç, F., Mat, A., (2019). Türkiye'de yanık tedavisinde geleneksel olarak kullanılan bitkiler. Sağlık Bilimlerinde İleri Araştırmalar Dergisi, 2(1), 15-35.
6. Demirezer, Ö., Saraçoğlu, İ., Şener, B., Köroğlu, A., Yalçın, F.N. (2017). FFD Monografları Bitkiler ve Etkileri. Akademisyen Kitabevi, Ankara.
7. Akinyele, B.O., Odiyi, A.C. (2007). Comparative study of vegetative morphology and the existing taxonomic status of *Aloe vera* L. Journal of Plant Sciences, 2(5), 558-563. [CrossRef]
8. Hamman, J.H. (2008). Composition and applications of *Aloe vera* leaf gel. Molecules, 13(8), 1599-1616. [CrossRef]
9. Misir, J., Brishti, F.H., Hoque, M.M. (2014). *Aloe vera* gel as a novel edible coating for fresh fruits: A review. American Journal of Food Science and Technology, 2(3), 93-97. [CrossRef]
10. Vázquez, B., Avila, G., Segura, D., Escalante, B. (1996). Antiinflammatory activity of extracts from Aloe vera gel. Journal of Ethnopharmacology, 55(1), 69-75. [CrossRef]

11. Tabandeh, M.R., Oryan, A., Mohammadalipour, A. (2014). Polysaccharides of *Aloe vera* induce MMP-3 and TIMP-2 gene expression during the skin wound repair of rat. International Journal of Biological Macromolecules, 65, 424-430. [CrossRef]
12. Barcroft, A., Myskja, A. (2003). Aloe vera: Nature's Silent Healer. BAAM, London.
13. Sari, A.O., Bilgin, O., Bilgiç, A., Nedret, T., Güvensen, A., Şenol, S.G. (2010). Ege ve Güney Marmara bölgelerinde halk ilacı olarak kullanılan bitkiler. Anadolu Ege Tarımsal Araştırma Enstitüsü Dergisi, 20(2), 1-21.
14. Rodriguez-Bigas, M., Cruz, N.I., Suarez, A. (1988). Comparative evaluation of *Aloe vera* in the management of burn wounds in guinea pigs. Plastic and Reconstructive Surgery, 81(3), 386-389. [CrossRef]
15. Fozzard, H.A., Sheets, M.F., Hanck, D.A. (2011). The sodium channel as a target for local anesthetic drugs. Frontiers in Pharmacology, 2, 68. [CrossRef]
16. Garutti, I., Rancan, L., Simón, C., Cusati, G., Sanchez-Pedrosa, G., Moraga, F., Olmedilla, L., Lopez-Gil, M.T., Vara, E. (2014). Intravenous lidocaine decreases tumor necrosis factor alpha expression both locally and systemically in pigs undergoing lung resection surgery. Anesthesia & Analgesia, 119(4), 815-828. [CrossRef]
17. Wen, F., Liu, Y., Wang, H., Tang, W., Hou, Y.D., Wang, H.L. (2017). Lidocaine inhibits the production of IL-1β from macrophages RAW264. 7 induced with lipopolysaccharide. International Journal of Clinical And Experimental Pathology, 10(6), 6582-6588.
18. Singh Malik, D., Mital, N., Kaur, G. (2016). Topical drug delivery systems: A patent review. Expert Opinion on Therapeutic Patents, 26(2), 213-228. [CrossRef]
19. Torin Huzil, J., Sivaloganathan, S., Kohandel, M., Foldvari, M. (2011). Drug delivery through the skin: Molecular simulations of barrier lipids to design more effective noninvasive dermal and transdermal delivery systems for small molecules, biologics, and cosmetics. Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology, 3(5), 449-462. [CrossRef]
20. Elias, P.M. (1988). Structure and function of the stratum corneum permeability barrier. Drug Development Research, 13(2-3), 97-105. [CrossRef]
21. Chang, R.K., Raw, A., Lionberger, R., Yu, L. (2013). Generic development of topical dermatologic products: formulation development, process development, and testing of topical dermatologic products. The AAPS Journal, 15(1), 41-52. [CrossRef]
22. Kumar, D., Jain, N., Gulati, N., Nagaich, U. (2013). Nanoparticles laden *in situ* gelling system for ocular drug targeting. Journal of Advanced Pharmaceutical Technology & Research, 4(1), 9. [CrossRef]
23. Madan, M., Bajaj, A., Lewis, S., Udupa, N., Baig, J.A. (2009). *In situ* forming polymeric drug delivery systems. Indian Journal of Pharmaceutical Sciences, 71(3), 242. [CrossRef]
24. Üstündağ Okur, N., Yoltaş, A., Yozgatlı, V. (2016). Development and characterization of voriconazole loaded *in situ* gel formulations for ophthalmic application. Turkish Journal of Pharmaceutical Sciences, 13(3), 311-317. [CrossRef]
25. Liu, L., Gao, Q., Lu, X., Zhou, H. (2016). *In situ* forming hydrogels based on chitosan for drug delivery and tissue regeneration. Asian Journal of Pharmaceutical Sciences, 11(6), 673-683. [CrossRef]
26. Peppas, N.A., Langer, R. (1994). New challenges in biomaterials. Science, 263(5154), 1715-1720. [CrossRef]
27. Ali Ibrahim, E.S., Ismail, S., Fetih, G., Shaaban, O., Hassanein, K., Abdellah, N H. (2012). Development and characterization of thermosensitive pluronic-based metronidazole *in situ* gelling formulations for vaginal application. Acta Pharmaceutica, 62(1), 59-70. [CrossRef]
28. Okur, M.E., Ayla, Ş., Batur, Ş., Yoltaş, A., Genç, E., Pertek, S., Üstündağ Okur, N. (2019). Evaluation of *in situ* gel containing pycnogenol for cutaneous wound healing. Medeniyet Medical Journal. 34(1), 67-75. [CrossRef]
29. Ur-Rehman, T., Tavelin, S., Gröbner, G. (2011). Chitosan in situ gelation for improved drug loading and retention in poloxamer 407 gels. International Journal of Pharmaceutics, 409(1-2), 19-29. [CrossRef]
30. Koşka, E.T. (2013). Master's Thesis. Administration and experimentation of polyacrylate based hydrogel microspheres for prolonging the activity period of lidocaine (2-diethylamino-n-(2,6 dimethylphenyl) acetamide) as a local anaesthetic. Department of Bioengineering, Faculty of Engineering, Hacettepe University, Ankara, Turkey.
31. Guideline IHT. (2005). Validation of analytical procedures: Text and methodology. Q2 (R1), 1(20), 05.
32. Chang, J.Y., Oh, Y.K., Choi, H.G., Kim, Y.B., Kim, C.K. (2002). Rheological evaluation of thermosensitive and mucoadhesive vaginal gels in physiological conditions. International Journal of Pharmaceutics, 241(1), 155-163. [CrossRef]

33. Liu, X., Gan, H., Hu, C., Sun, W., Zhu, X., Meng, Z., Dou, G. (2019). Silver sulfadiazine nanosuspension-loaded thermosensitive hydrogel as a topical antibacterial agent. International Journal of Nanomedicine, 14, 289. [CrossRef]
34. Ijaz, N., Durrani, A.I., Rubab, S., Bahadur, S. (2022). Formulation and characterization of *Aloe vera* gel and tomato powder containing cream. Acta Ecologica Sinica, 42(2), 34-42. [CrossRef]
35. Ishihara, M., Nakanishi, K., Ono, K., Sato, M., Kikuchi, M., Saito, Y., Yura, H., Matsui, T., Hattori, H., Uenoyama, M., Kurita, A. (2002). Photocrosslinkable chitosan as a dressing for wound occlusion and accelerator in healing process. Biomaterials, 23(3), 833-840. [CrossRef]
36. Mohamad, N., Amin, M.C.I.M., Pandey, M., Ahmad, N., Rajab, N.F. (2014). Bacterial cellulose/acrylic acid hydrogel synthesized via electron beam irradiation: Accelerated burn wound healing in an animal model. Carbohydrate Polymers, 114, 312-320. [CrossRef]
37. Balakrishnan, B., Mohanty, M., Umashankar, P.R., Jayakrishnan, A. (2005). Evaluation of an in situ forming hydrogel wound dressing based on oxidized alginate and gelatin. Biomaterials, 26(32), 6335-6342. [CrossRef]
38. Hubbell, J.A. (1996). Hydrogel systems for barriers and local drug delivery in the control of wound healing. Journal of Controlled Release, 39(2-3), 305-313. [CrossRef]
39. Rehman, K., Zulfakar, M.H. (2014). Recent advances in gel technologies for topical and transdermal drug delivery. Drug Development and Industrial Pharmacy, 40(4), 433-440. [CrossRef]
40. Stanciauskaite, M., Marksa, M., Ivanauskas, L., Perminaite, K., Ramanauskiene, K. (2021). Ophthalmic *in situ* gels with balsam poplar buds extract: Formulation, rheological characterization, and quality evaluation. Pharmaceutics, 13(7), 953. [CrossRef]
41. Joshi, S.C. (2011). Sol-gel behavior of hydroxypropyl methylcellulose (HPMC) in ionic media including drug release. Materials, 4(10), 1861-1905. [CrossRef]
42. Wang, M., Xu, L., Hu, H., Zhai, M., Peng, J., Nho, Y., Wei, G. (2007). Radiation synthesis of PVP/CMC hydrogels as wound dressing. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms, 265(1), 385-389. [CrossRef]
43. Iwu, M.M. (1993). Handbook of African medicinal plants. CRC Press, Maryland, p.183-184.
44. Aksu, N.B., Yozgatlı, V., Okur, M.E., Ayla, Ş., Yoltaş, A., Okur, N.Ü. (2019). Preparation and evaluation of QbD based fusidic acid loaded *in situ* gel formulations for burn wound treatment. Journal of Drug Delivery Science and Technology, 52, 110-121. [CrossRef]
45. Joshi, N., Mishra, N., Rai, V.K. (2021). Development and evaluation of in situ gel of silver sulfadiazine for improved therapeutic efficacy against infectious burn wound. Journal of Pharmaceutical Innovation, 16(3), 537-550. [CrossRef]
46. Majeed, A., Khan, N.A. (2019). Ocular in situ gel: An overview. Journal of Drug Delivery and Therapeutics, 9(1), 337-347. [CrossRef]
47. Proksch, E. (2018). pH in nature, humans and skin. The Journal of Dermatology, 45(9), 1044-1052. [CrossRef]
48. Aydin E. (2013). Master's Thesis. Development of a ocular nanosystem formulation containing a nonsteroidal anti-inflammatory drug. Department of Pharmaceutical Technology, Faculty of Pharmacy, Ankara University, Ankara, Turkey.
49. Okur, N.Ü., Yozgatli, V., Şenyiğit, Z. (2020). Formulation and detailed characterization of voriconazole loaded *in situ* gels for ocular application. Journal of Faculty of Pharmacy of Ankara University, 44(1), 33-49. [CrossRef]
50. Owczarz, P., Rył, A., Wichłacz, Ż. (2019). Application of texture profile analysis to investigate the mechanical properties of thermosensitive injectable chitosan hydrogels. Progress on Chemistry and Application of Chitin and its Derivatives, 24, 151-163. [CrossRef]
51. Patlolla, V.G.R., Peter Holbrook, W., Gizurarson, S., Kristmundsdottir, T. (2019). Doxycycline and monocaprin *in situ* hydrogel: Effect on stability, mucoadhesion and texture analysis and *in vitro* release. Gels, 5(4), 47. [CrossRef]
52. Koffi, A.A., Agnely, F., Ponchel, G., Grossiord, J.L. (2006). Modulation of the rheological and mucoadhesive properties of thermosensitive poloxamer-based hydrogels intended for the rectal administration of quinine. European Journal of Pharmaceutical Sciences, 27(4), 328-335. [CrossRef]