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PREPARATION AND CHARACTERIZATION OF Ibuprofen LOADED ALOE VERA GEL FORMULATION

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ABSTRACT:

Objective: This study used gel derived from the aloe vera plant and the active component Ibuprofen to create topically applied gel compositions intended to modulate anti-inflammatorys. Higher drug residence time was achieved by the generated in situ gels, which were in liquid state at room temperature, gelled at body temperature, and stuck to the wound surface. It is hoped that by enhancing the in situ gels' distinguishing features, a greater local Ibuprofen concentration will increase patient compliance.

Material and Method: Using characteristic analyses, in situ gel formulations that were divided by assigning distinct gel codes were looked at. Measurements of the gelation temperature, pH, in vitro release of Ibuprofen, viscosity and rheological characteristics, and the textural profile of the formulations were all done as part of these analyses.

Result and Discussion: It has been demonstrated that poloximer 407-based in situ gels with Ibuprofen and aloe vera gel for topical treatment lengthen the duration of skin residence. The formulations designated F5 and A21 exhibited a gelation temperature suitable for topical application and a sustained release of Ibuprofen for a full day, despite the formulations containing varying percentages of the polymers Poloxamer 407, Poloxamer 188, HPMC, and CMC. These results suggest that an in situ gel formulation based on poloxamer 407-HPMC may be a useful substitute for topical anti-inflammatory drugs.

Keywords: Aloe vera, anti inflammatory treatment, in situ gel, Ibuprofen, topical formulation treatment.

INTRODUCTION :

anti inflammatory 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]. anti inflammatory injuries are the fourth most common trauma sources around the world [3].

In treatment of anti inflammatory 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 anti inflammatory wound sepsis and its related morbidity and mortality [4]. In addition to clinical treatment, medicinal plants take a significant role in the healing of anti inflammatory 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 anti inflammatory wounds with different anti inflammatory degrees. This positive effect may be resulted from the antimicrobial, anti-inflammatory, antioxidant, astringent, collagen synthesis stimulatorand 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, anti inflammatorys and wound healing properties of topical *Aloe vera* application in gel form have also been reported [10,11]. It is also used in treatmentof 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 anti inflammatorys, sunanti inflammatorys and X-ray anti inflammatorys [6]. The anti-inflammatory property of the *Aloe vera* contributes to the improvement of the inflammatoryprocess caused by the anti inflammatory injury. Since blocking the formation of vasoactive prostanoids prevents vasoconstriction thrombosis and the progressive ischemic necrosis known to ocur in thermal and electric

anti inflammatorys 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 anti inflammatory 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]. Ibuprofen 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 theirapplication in a variety of biomedical fields, including drug delivery [26].

MATERIAL AND METHOD

Chemicals

Ibuprofen (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 andhomogenized 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. Ibuprofen 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 Ibuprofen

Analytic validation by UV for Ibuprofen 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 (WTWProfi 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. *Insitu* 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 Ibuprofen and *Aloe vera*, the cold method was used. *Aloe vera* was preferred due to anti-inflammatory, wound and anti inflammatory 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.Gel formulation

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

Code	P407 (%)	HPMC (%)	*Aloe vera* gel (%)	Ibuprofen (%)	Benzalkonium chloride (%)	Sol-Gel Temp. (±0.5°C, n=3)
F1	18	0.5	-	5	0.01	30.5°C
F2	18	1	-	5	0.01	32.8°C
F3	18	2.5	-	5	0.01	Solid Below 5°C
F4	18	5	-	5	0.01	Solid Below 5°C
F5	20		-	5	0.01	35.3°C
F6	20	0.5	-	5	0.01	28.8°C
F7	20	1	-	5	0.01	28.3°C
F8	20	2.5	-	5	0.01	Solid Below 5°C

F9	20	5	-	5	0.01	Solid Below 5°C
F10	22	0.5	-	5	0.01	25.5°C
F11	22	1	-	5	0.01	25.6°C
F12	22	2.5	-	5	0.01	Solid Below 5°C
F13	22	5	-	5	0.01	Solid Below 5°C
A1	18	0.5	10	5	0.01	31.8°C
A2	18	0.5	20	5	0.01	Liquid Above 50°C
A3	18	0.5	25	5	0.01	Liquid Above 50°C
A4	18	1	10	5	0.01	Liquid Above 50°C
A5	18	1	20	5	0.01	Liquid Above 50°C
A6	18	2.5	10	5	0.01	Solid Below 5°C
A7	18	2.5	20	5	0.01	Solid Below 5°C

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

Code	**P407 (%)**	**HPMC (%)**	***Aloe vera* gel (%)**	**Ibuprofen (%)**	**Benzalkonium chloride (%)**	**Sol-Gel Temp. (□0.5°C, n=3)**
A8	18	2.5	50	5	0.01	Solid Below 5°C
A9	18	5	10	5	0.01	Solid Below 5°C
A10	18	5	20	5	0.01	Solid Below 5°C
A11	18	5	50	5	0.01	Solid Below 5°C
A12	20	0.5	10	5	0.01	Liquid Above 50°C
A13	20	0.5	20	5	0.01	Liquid Above 50°C
A14	20	0.5	25	5	0.01	Liquid Above 50°C
A15	20	1	10	5	0.01	31.8°C
A16	20	1	15	5	0.01	Liquid Above 50°C
A17	20	1	20	5	0.01	Liquid Above 50°C
A18	20	1	25	5	0.01	Liquid Above 50°C
A19	20	5	50	5	0.01	Solid Below 5°C
A20	22	0.5	10	5	0.01	28.5°C
A21	22	0.5	20	5	0.01	36.5°C
A22	22	0.5	25	5	0.01	Liquid Above 50°C
A23	22	1	10	5	0.01	27.5°C
A24	22	1	20	5	0.01	31.8°C
A25	22	1	22.5	5	0.01	33.3°C

A26	22	1	25	5	0.01	Liquid Above 50°C
A27	22	5	10	5	0.01	Solid Below 5°C
A28	22	5	20	5	0.01	Solid Below 5°C
A29	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 Ibuprofen 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 Ibuprofen 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 (%) recoveryof samples were found to be 95.929%.

pH values of F_5 and A_{21} formulations prepared with Ibuprofen 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.

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

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

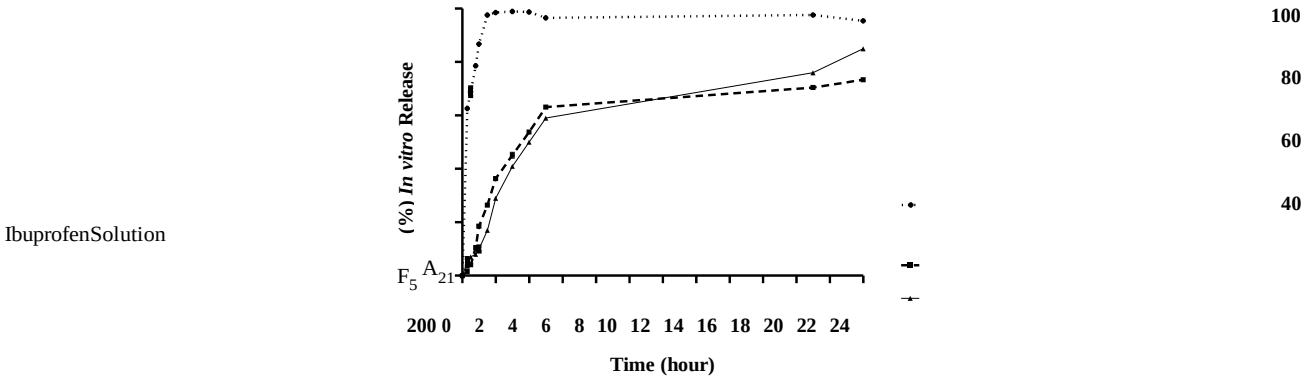

Figure 4. *In vitro* release profiles of Ibuprofen solution, F5 and A21 *in situ* gel formulations(Mean ± SD; n = 3)

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

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 sunanti inflammatorys, X-ray anti inflammatorys, thermal and electrical anti inflammatorys have been proven in various literature [43].

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

For partial validation of the analytical method for the quantification of Ibuprofen 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 Ibuprofen showed maximum absorbance. This data shows that the quantification method is specific to Ibuprofen 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 preparedwere 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; withthis 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, thehardness 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 $F_{\mathbf{5}}$ gel and lower in $A_{\mathbf{21}}$ gel. HPMC and poloxamer polymers show mucoadhesive properties. However, although the polymer concentration is

higher in the $F_{\mathbf{5}}$ 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 Ibuprofen solution (Figure 4). At the same time, the Ibuprofen and *Aloe vera* loaded *in situ* gel might display a higher anti inflammatory wounds therapy effect compared with the conventional Ibuprofen solution due to quick andconstant drug release profile.

In conclusion, Ibuprofen and *Aloe vera* containing *in situ* gel formulations were developed and characterized for treatment of anti inflammatory 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. $F_{\mathbf{5}}$ and $A_{\mathbf{21}}$ 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* Ibuprofen release for 24 hours. According to these results Ibuprofen and *Aloe vera* containing Poloxamer 407-HPMC based *in situ* gel formulations can be concluded as an effective alternative for topical treatment of anti inflammatory wounds.

CONFLICT OF INTEREST

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

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