

## Gelation of Sodium-Deoxycholate: Influenced by the Presence of a Corticosteroid-Betamethasone-17-Valerate

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### Abstract

This study was designed to evaluate the possible influences of betamethasone-17-valerate (BMV) on the gelation properties of sodium deoxycholate (Na-DOC), due to structural similarities as the monomer and the active agent. For this purpose gel formulations at two different concentrations, 0.05 and 0.1 % (w/w) were prepared and characterized by rheological and thermal analysis. The results indicated that the elastic moduli of the gels increased with increasing concentration of BMV in the system. This phenomenon was shown to be due to the modification of polymerization occurred by interactions between BMV and Na-DOC. The increment of BMV concentration in the formulation, caused a modification of the glass transition temperature ( $T_g$ ) detected.  $T_g$  data pointed out a higher polymerization of Na-DOC with increasing amount of BMV. Transepidermal water loss measurements were also performed to evaluate possible skin irritation. It was observed that the degree of Na-DOC polymerization did not show any irritant effect on the skin.

Overall, the results of this study clearly confirmed that incorporation of BMV into Na-DOC gel system produced a synergistic effect on product rheological characteristics, which would be beneficial for topical applications.

**Keywords:** Betamethasone valerate, Sodium-deoxycholate, Gel, Rheology, Transepidermal water.

### Introduction

Sodium deoxycholate (Na-DOC), was proposed as an alternative to polymeric matrices for developing drug carrier systems [1-3]. Na-DOC is a low molecular weight polymer (414.5) with advantages as low melt viscosity, potential biocompatibility, biodegradability and the absence of toxic impurities [4] (Valenta et al., 1999). Na-DOC has been used as a penetration enhancer in various administration routes [5-7] and in our previous study Na-DOC has been optimized for the improvement of the skin delivery of betamethasone-17-valerate (BMV) [8]. BMV is the gold standard of topical corticosteroids and it is used as a reference in the clinical studies for the registration of new glucocorticosteroids [9]. BMV is a medium potency glucocorticoid, and it

can be found as topical dosage forms like cream, ointment, lotion and foam all with a strength at 0.1 % w/w of betamethasone base [10]. In our previous study the enhanced in vitro flux and higher in vivo pharmacodynamic responses in rats were determined, in comparison with a commercial product of BMV [8].

Na-DOC is able to form gels due to the formation of the helical macromolecular complex from the bile acid steroid when in contact with excess buffer system [4,8]. BMV has a steroid ring also and it is susceptible to an acid and base catalyzed isomerization in aqueous and semi-solid formulations resulting in an acyl group migration from position C17 to the more stable C21 position of the steroid ring system [11].

Therefore, this study was designed to evaluate the possible influence of BMV on the gelation properties of Na-DOC. Since the monomer and the active agent, both contained a steroid ring, the effect of drug concentration on the gelation phenomena of Na-DOC were investigated. The characterizations were conducted by means of flow and oscillatory rheometry and frequency sweep analysis. Glass transition temperature ( $T_g$ ) were measured by differential scanning calorimetry (DSC) to evaluate the degree of polymerization. Furthermore, transepidermal water loss (TEWL) measurements were conducted to explore possible skin irritation properties of the formulations with different polymerization degree, in comparison with a topical commercial cream.

### Methods

#### Materials

Micronized betamethasone-17-valerate (BMV) was a kind gift from GlaxoSmithKline (Turkey). Na-DOC and mannitol were purchased from Fluka (Germany) and Merck (Germany), respectively. All other chemicals were of analytical grade.

#### Preparation of Gel Formulations

0.5 % Na-DOC was dissolved in phosphate buffer saline (PBS: 0.1 M phosphate buffer (pH=7.2) containing 0.9 % sodium chloride) and 5% of mannitol was added [4]. BMV was added into these formulations in two different amounts (0.05 and 0.1 %, coded as F1 and F2, respectively) under continuous stirring.

## Rheological Analysis

Rheological analyses were performed by a Haake rheometer (Haake Mars Modulars Advanced Rheometer Systems, Germany) equipped with thermostatically-controlled cone/plate geometry with 60 mm diameter and 1° angle, in triplicate. The cone/plate temperature was kept at 5°C during the study.

### Rheologic behavior and dynamic viscosity determination

Upward and downward flow curves for formulations were evaluated between shear rates of 0.001–8900 s<sup>-1</sup> in continuous shear analysis. Shear rate was increased over a period of 150 s, held at the upper limit for 10 s, and then decreased over a period of 150 s. Each measurement was replicated three times.

The flow characteristics were observed at the 0.05–10 Pa range of stress at 5°C. Viscosities of non-Newtonian systems were recovered from the beginning of the flow curves. And “Power Law”:  $\sigma = K \cdot \dot{\gamma}^n$  was used to analyse the data ( $\sigma$ : shear stress,  $K$ : consistency index,  $\dot{\gamma}$ : shear rate,  $n$ : power law index).

A straight line in a shear rate/shear-stress plot represents the shearing behaviour, and gives an opportunity for the approximation of the shearing properties of fluids. For Newtonian samples the power law model gives  $n = 1$  and  $K = 1$ , for a shear thinning (pseudo-plastic) fluid  $n < 1$  and for a shear thickening (dilatant) fluid  $n > 1$  [12].

### Oscillation stress sweep

The sample was exposed to increasing stress at a constant frequency; at 5°C, 1Hz frequency and at the stress range of 0.1–1000 Pa. The  $G$  values were in logarithmic scale. The linear viscoelastic regime (LVR) of the sample was determined, the stress value to use in the other oscillation tests was chosen.

### Frequency sweep

The sample was exposed to a stepwise of increasing frequency at a constant stress (0.5 Pa); 0.01–10 Hz frequency range, in the field of linear viscoelasticity, at 5°C. The shear strain, the stress and the phase angle were determined from oscillating measurements. The parameters obtained were the complex modulus,  $G^*$ , and the phase angle  $\delta$ . The elastic modulus ( $G'$ ), the viscous modulus ( $G''$ ) and the dynamic viscosity ( $\eta$ ) were calculated using following equations:

$$G^* = G' + iG'' \quad (1)$$

$$G' = G^* \cos(\delta) \quad (2)$$

$$G'' = G^* \sin(\delta) \quad (3)$$

$$\dot{\eta} = G''/\omega \quad (4)$$

Where,  $\omega$  is the angular frequency, which was varied from 0.01 to 100 Hz. In each case, the dynamic rheological properties were determined with at least three replicates.

### Thermal Analysis

Differential scanning calorimetric (DSC) analysis was performed by a Perkin Elmer, DSC-8000 to determine melting points and  $T_g$ . The samples were sealed in aluminum pans under nitrogen air atmosphere at a flow rate of 20 ml/min and evaluated in 20–250 °C temperature ranges. BMV, Na-DOC, physical mixture of Na-DOC: BMV (1:1), Na-DOC gel (BMV free), F1 and F2 formulations were evaluated.

## Transepidermal Water Loss Measurements

Male Albino Wistar rats, weighing 180–220 g were housed in standard environmental conditions and fed with standard rodent diet with water *ad libitum*. The experimental protocol was approved by the Local Animal Ethical Committee of Ege University, Faculty of Pharmacy (Approval No. 2007/2-1).

To determine transepidermal water loss (TEWL) produced on the skin after the contact of Na-DOC gels, twenty-four rats divided into four groups as one being the control. Before TEWL measurements, rats were anesthetized by intraperitoneal injection of ketamine (30 mg/kg). After recording initial TEWL measurements, 0.5 g of formulation (Na-DOC gel or commercial cream) was spread uniformly on 2x2 cm<sup>2</sup> area of shaven rat abdomen and left for 1 hour. TEWL measurements were performed during 1 minute using a Tewameter TM 300 (Courage + Khazaka electronic GmbH, Germany) 5 min, 2 h and 4 h after removal of the formulations (done by cotton wiping). The laboratory temperature and humidity were kept constant in the range of 24–25°C and 45–50 %, respectively.

### Statistical Analysis

Statistical differences were determined using Repeated Measures ANOVA test. Significance was determined by Bonferroni test as a post hoc test. Data were considered significant at  $p < 0.05$ .

## Results and Discussion

In this study the detailed rheological analysis and thermal characterizations were performed on both the blank and BMV containing Na-DOC gels. In our previous study, the flow properties of just blank Na-DOC gels were reported [8]. The results of oscillation stress sweep tests indicated that blank and BMV incorporated gels showed a linear viscoelastic regimen (LVR) up to 0.6 Pa therefore the tests were conducted at 0.5 Pa. The rheological studies assessing viscosity at a defined shear rate showed that BMV incorporated Na-DOC-gels display thixotropic behavior, similar to blank formulations. Hyteresis loop- the up-down curve of thixotropic systems can be seen in Figure 1.

Thixotropy is defined as the property of some non-Newtonian pseudoplastic fluids to show a time-dependent change in viscosity. As can be seen from Figure 1 the longer the gel underwent shear, the lower was its viscosity. All of the gel formulations were viscous under normal conditions, but flow under a certain pressure. This kind of flow was addressing a reversible gel-sol-gel transformation. Upon setting, a network was formed again and provided a rigid matrix that stabilized the gels. In thixotropic preparations, the systems are made ready to flow by tapping or spreading [13,14]. This property of Na-DOC gels might be used as an advantage due to the ease of application of dermatocorticosteroids.

In our study, BMV was found to modify the viscosity of gels and this modification was concentration dependent. The viscosity of the system increased when the concentration of BMV was increased in the formulation as 2 folds (0.1% the upper dose limit that is allowed). It is clear from Figure 2 that in all shear rate ranges (8900-200 Hz, 200-20 Hz and 20-1 Hz), gels displayed significantly different viscosity profiles ( $P < 0.05$ ). The highest viscosity in all shear ranges were obtained with 0.1% BMV

incorporated gels even at the highest shear range of 8900-200 Hz. When we consider viscosity of 0.1% BMV gels at the lowest shear rates, the values were dramatically different, such as 140 folds in comparison to blank gels (Table 1).

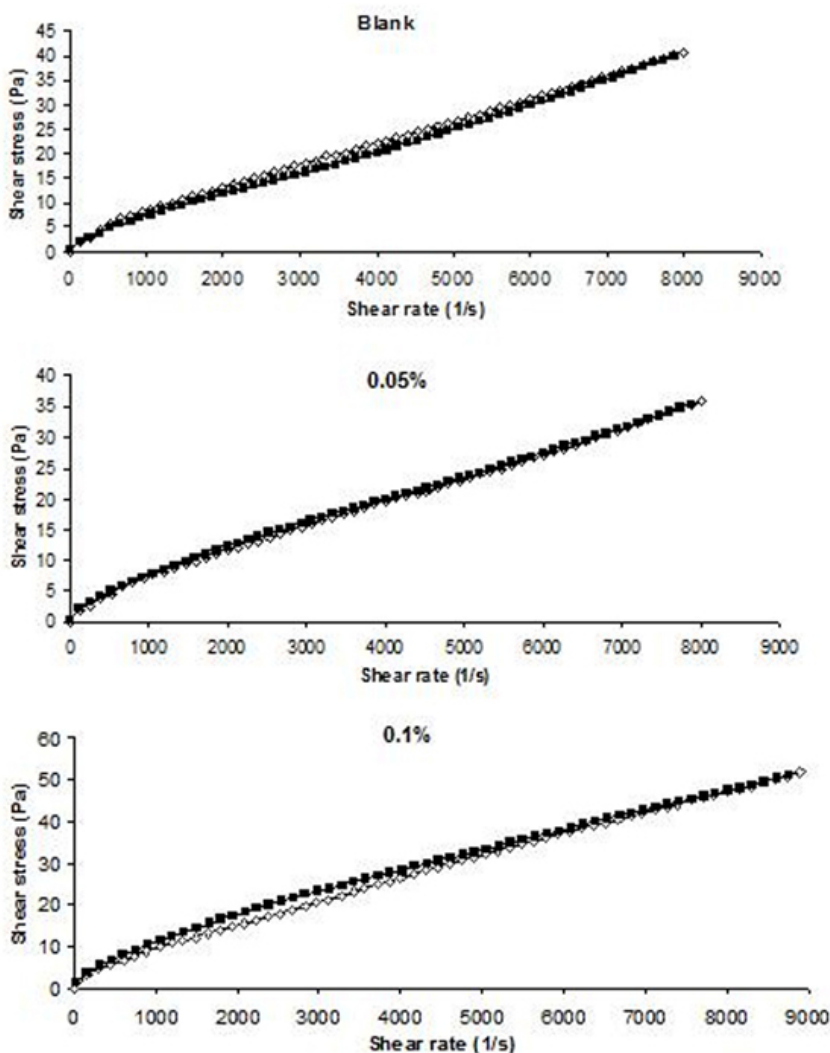
**Table 1:** Viscosity Values of Formulations in Different Shear Rates

Shear Rate [1/s]	blank	0.05%	0.1%
0.92	22.12	634.1	1068
0.442	38.11	1014	1683
0.213	59.85	1589	2580
0.102	94.12	2226	3973
0.0488	150.6	3129	5978
0.02373	229.8	4194	9993
0.01124	308.4	5844	14070
0.004597	323.4	6981	23400
0.002725	323.4	8880	32460
0.001038	323.4	10740	46400

This behavior was attributed to the hydrogen-bonds forming inside Na-DOC gel system. Na-DOC aggregates under suitable conditions resulting as a gel formed by helical macromolecules. Na-DOC is a steroid with C-24 carboxylate group (Figure 3a) and

joins to a polar head group and like bile salts and forms small micelles. Micelle growth in bile salts is documented by a lot of experimental evidence, based on viscosity and hydrogen bonding was noted as the driving force in the micellar aggregation process [15,16]. Such data pointed out the existence of a continuous process driven by the electrostatic repulsions [17]. The more the steroid ring was present in the system, the more the gelation occurred. The increment of BMV concentration had a significant effect on the gelation phenomena of the Na-DOC indicated by an increased viscosity.

Thermal analysis studies were conducted to understand the polymerization process. The concept of  $T_g$  is very important in determining the performance and potential implementation of a polymer network since it is a function of chain flexibility. The glass transition takes place when thermal energy in the system is enough to create sufficient free-volume to let 6-10 main-chain carbons to move together as a unit. The molecular chains slide past each other when a force is applied. However the introduction of chemical groups, like steroid rings, will interfere with the flowing process, hence increase viscosity and  $T_g$  [18]. Moreover, the enhanced hydrogen bonds formed in Na-DOC gels might be the cause of enhanced  $T_g$  values. In a study, it was shown that hydrogen bond increment in the polymeric



**Figure 1:** Thixotropy graph of Na-DOC gels. Open symbol represents upcurve and closed symbol represents downcurve. Standard deviations have been omitted for clarity

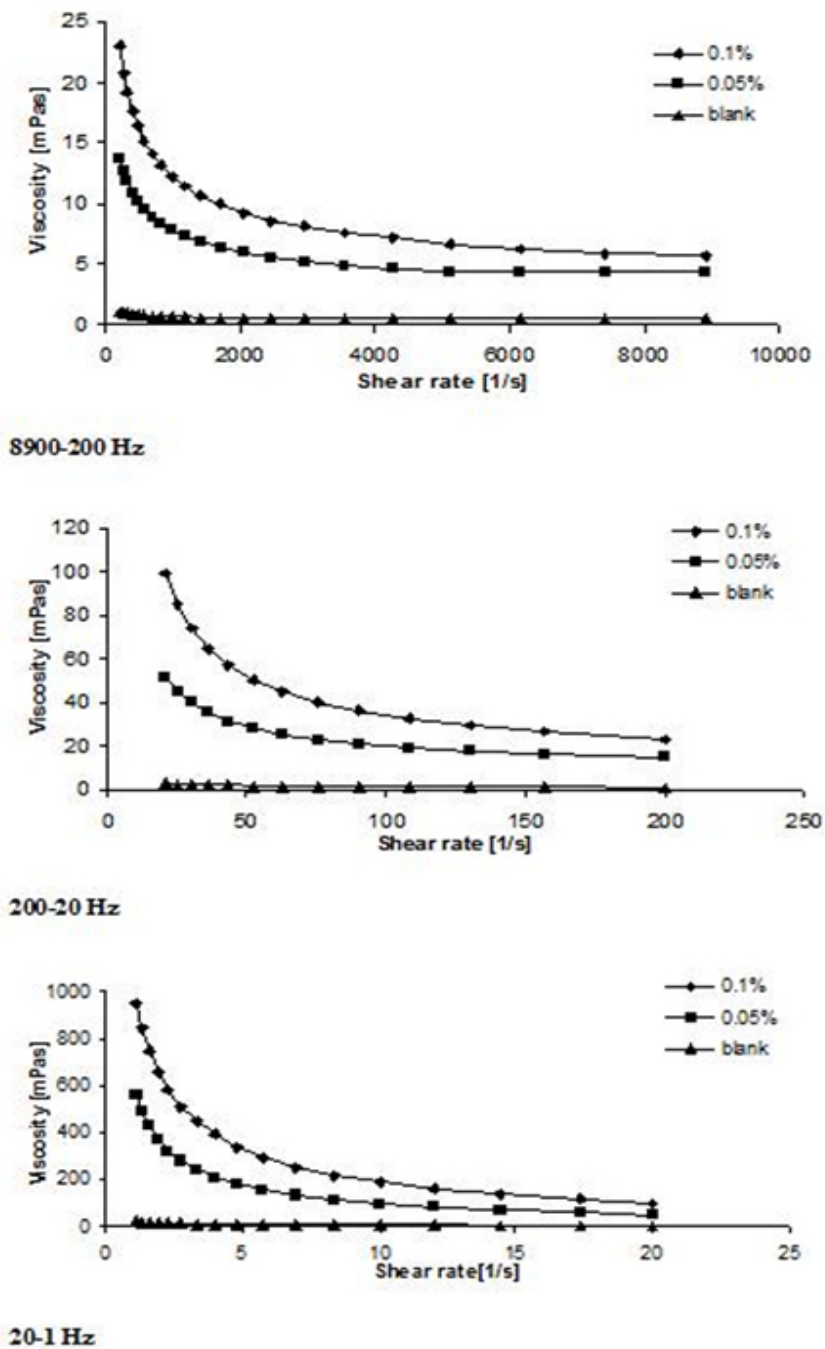


Figure 2: Viscosity profiles of formulations in different shear rates

system led  $T_g$  increment, resulting in the improved thermal stability [19]. In our study, thermal analysis was conducted via determining melting points and  $T_g$ .

The melting points of BMV and Na-DOC were determined as 200.18 °C and 153.71 °C, respectively. The sharp melting peak of Na-DOC disappeared when network has been formed (Figure 4). This data acted as an evidence of monomer polymerization.  $T_g$  values were compared to evaluate the polymerization degree of Na-DOC and to investigate the effect of BMV concentration in the gels (Figure 4). The BMV concentration in the network system strongly affected the  $T_g$  behaviour. Clearly,  $T_g$  of Na-DOC gel with higher BMV content was substantially greater than other compositions ( $T_{g_{\text{blank gel}}} 128.28 \text{ °C} < T_{g_{F1}} 146.10 \text{ °C} < T_{g_{F2}} 180.86 \text{ °C}$ ). It is known that polymerization is strongly

influenced by both the chemical structure of the monomers and the crosslink density of the resulting polymer. By adding a similar steroidal structure as an active substance (Figure 3b) in Na-DOC system, it might be possible to elevate the amount of interactive hydrogen bonds. Therefore the increment in the viscosity of BMV incorporated Na-DOC gels recorded in our study, might be attributed to the increment of the hydrogen bonding due to the structural similarity between BMV (Figure 3b) and Na-DOC (Figure 3a).

After determining the effect of BMV on viscosity and polymerization, elastic modulus ( $G'$ ) and viscous modulus ( $G''$ ) were monitored to define the rheological characteristics of formulations. The elastic (or storage) modulus represents the elastic storage of energy and it is a measure of a well-structured

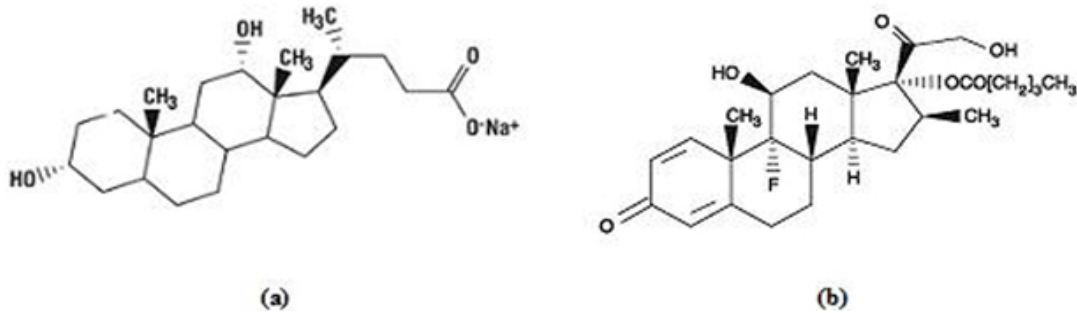


Figure 3: Molecular formulas of sodium-deoxycholate (a) and betamethasone-17-valerate (b)

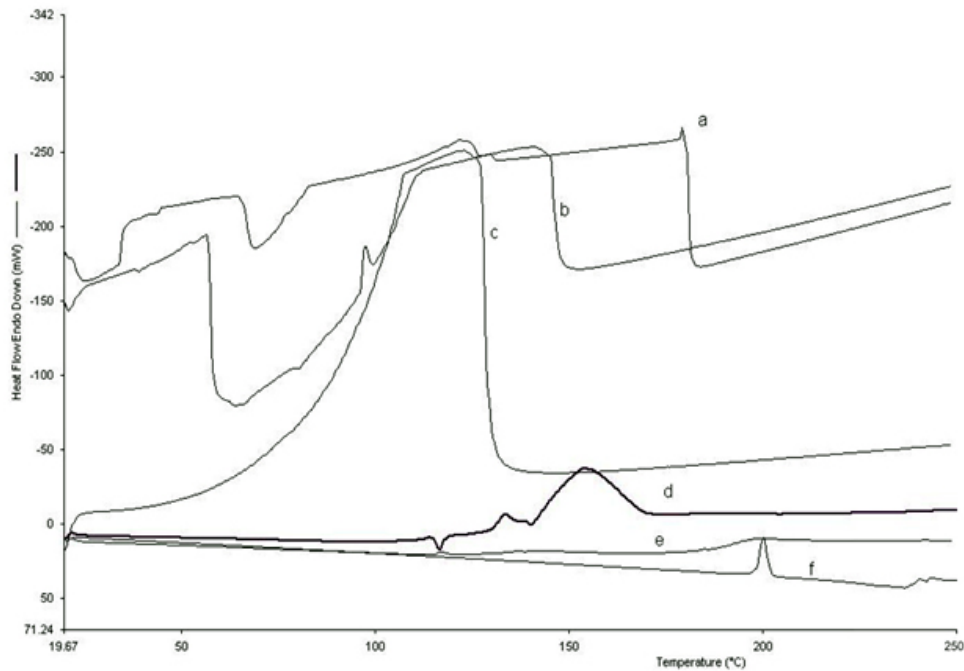


Figure 4: DSC thermograms of F2 (a), F1 (b), blank gel (c), Na-DOC (d), physical mixture (1:1) (e) and BMV (f)

material. The viscous (or loss) modulus represents the viscous energy dissipation and it will be large when the sample is predominantly viscous [20].

In this study, frequency sweep tests performed on blank Na-DOC gel systems (Figure 5) indicated a viscous fluid behavior instead of a well structured gel matrix. In the presence of a gel structure,  $G'$  modulus has to be greater than  $G''$  modulus in the frequency range examined and both moduli have to be frequency independent [21,22]. However for blank Na-DOC systems, the viscous property was dominant than the elastic property at the whole frequency range points ( $G'' > G'$ ).

Unfortunately, 0.05 % BMV incorporated system also shown a viscoelastic fluid behavior instead of a well-structured gel matrix. The viscous modulus  $G''$  was higher at the most of the frequency range. In comparison to the blank gel, the elastic properties were slightly improved due to elevated  $G'$  values. Nevertheless, the system still was not performing enough resistance to be defined as a gel (Figure 6).

However the duplication of BMV amount in Na-DOC system (0.1%), made a significant effect on the frequency behavior compared to blank system. The addition of higher amount of drug to the system, shifted the crossover value (when  $G'$

becomes equal to  $G''$ ) towards relatively higher frequencies as 2.7 Hz (Figure 7). As can be seen from Figure 6, in case of 0.05% BMV formulations, the crossover point has been below 1 Hz. The reciprocal of the frequency at the crossover point is regarded as the relaxation time of entangled network in the polymer solution [23]. The rheological studies indicated that, with incorporation of 0.1% BMV to the system, the gelation properties of Na-DOC could be improved.

The mechanical spectrum in the available frequency range of 0.1% BMV gel confirmed an increase in sample elasticity and a greater predominance of its elastic character. The typical rheological behavior of a gel structure was confirmed pointing out the absence of frequency dependence.

#### In Vivo Studies: Evaluation of TEWL and Skin Irritation

0.1% BMV content in Na-DOC system was found to be beneficial for improved gelation properties. However, possible skin irritation had to be addressed, in order to define formulation safety. It has been shown that, in rat skin, TEWL increase could be correlated to barrier function impairment produced by irritants [24]. In the study of Kolbe et al[25], it was shown that the treatment of a commercial cream of 0.05 % of Clobetasol propionate for 3-4 weeks resulted in the thinning of the epidermis and enhanced

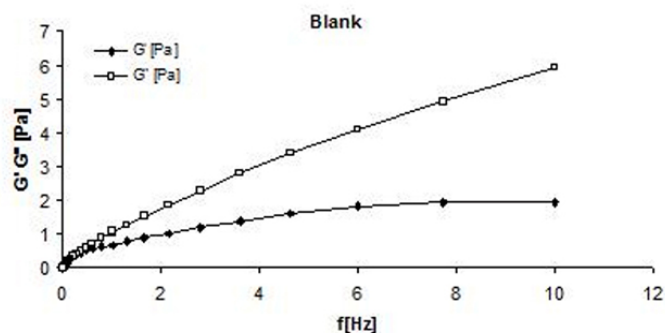


Figure 5: The elastic and viscous modulus plotted versus frequency for blank gel

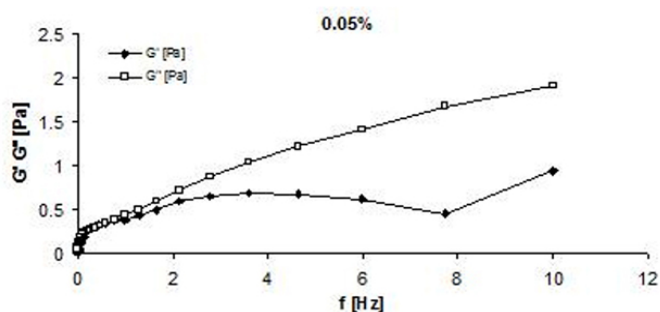


Figure 6: The elastic and viscous modulus plotted versus frequency for F1

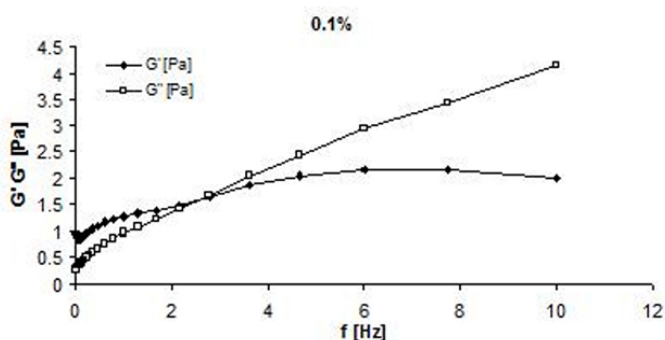


Figure 7: The elastic and viscous modulus plotted versus frequency for F2

transepidermal water loss. To assess a possible skin irritation produced by the formulations applied, TEWL measurements were performed in comparison to a commercial product; Betnovate<sup>®</sup> Cream (GlaxoSmithKline).

Typical basal values of TEWL obtained in the present work were determined between 11.3-12.8 g m<sup>-2</sup> h<sup>-1</sup>, close to the literature data reported before [24]. The TEWL value of rat skin is typically higher than the value of human skin (5-6 g m<sup>-2</sup> h<sup>-1</sup>), which may be due to the high density of hair follicles in Wistar rat skin [26,27]. Figure 8 shows the variation of TEWL before the application of the formulations and 5 min, 2 h and 4 h after its removal (total application time was 1 hour). For all formulations tested, TEWL increased in a significant way after 5 min and then decreased to reach the basal value after 4 hours. The reason for the alteration of TEWL after formulation removal was probably the evaporation of residues of water contained in the formulation from the skin surface. At the end of 5 min, TEWL values obtained from 0.1% BMV incorporated gel (F2) applied rats, were higher than commercial cream and 0.05% BMV having formulation. In our previous study it was shown that, the release of drug from 0.1%

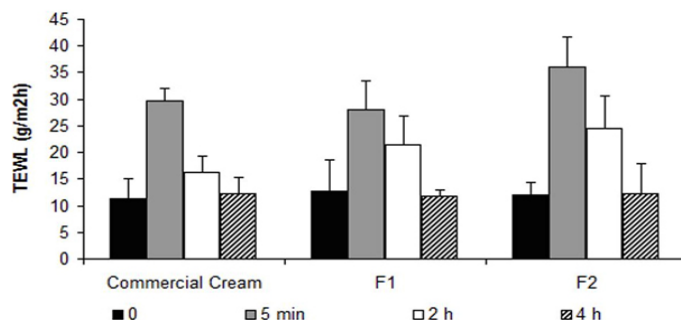


Figure 8: TEWL changes before and after removal at 5 min, 2 h and 4 h of indicated formulations

BMV gel was significantly higher than (approximately 20 fold) commercial gel [8]. This might explain relatively lower values obtained with commercial cream. As the release was higher, TEWL has become more predominant. However for time points of 2 h and 4 h there were no significant difference between formulations. Overall, at least for the time period examined in this work, the commercial cream and the gels prepared could be defined as non-irritant to rat abdominal skin since TEWL values have returned to basal values.

## Conclusion

In this study, the effect of BMV concentration on Na-DOC gel system were evaluated regarding rheological, thermal and TEWL properties. The BMV presence in the formulations acted as an enhancer of polymerization determined by *T<sub>g</sub>* data. Moreover rheological studies conducted in Na-DOC systems with a higher BMV content (0.1%), confirmed an increase in sample elasticity. The greater predominance of its elastic character pointed out the improved gelation. According to the results of TEWL studies, the prepared formulations could be defined as non-irritant on the skin.

In conclusion, we can state that BMV addition to Na-DOC system modifies the polymerization in favor of gelation phenomenon without any important skin irritation.

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