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Research Article

Hydrogel Formulation of *Buchanania lanzan* Spreng-A Focus on Rheological Properties

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ABSTRACT
The aim of this study was to characterize and optimize the different hydrogel formulations of the methanolic root extract of *Buchanania lanzan* (MEBL) with an objective towards the development of a suitable topical delivery system. The gel formulations were prepared with different concentrations of polymer (Carbopol-940) and 0.5% of the active fraction (ethyl acetate sub-fraction of MEBL). Different hydrogel formulations were analyzed for rheological properties, spreadability, homogeneity and stability. A comparative study of rheological parameters, and spreadability showed that the gel formulations displayed all the desirable properties, which are considered to be essential prerequisites for a standard stable gel formulation. Based on the rheological studies of all the formulations, sample F3 was found to be a stable formulation with comparatively superior rheological characteristics.

Keywords: *Buchanania lanzan*, Hydrogels, Rheology, Stability

INTRODUCTION

Topical drug delivery systems are an attractive route for local and systemic treatment of various skin diseases. The delivery of drugs onto the skin is recognized as an effective means of therapy for the management of different dermatological diseases. These preparations can penetrate deeper into skin and hence give better absorption [1]. A gel is a semisolid system of at least two interpenetrating phases: a gelling agent and a liquid. Gels that contain water are called hydrogels, while those that contain an organic liquid are referred as organogels. Hydrogels are a relatively newer class of dosage form created by entrapment of large amounts of aqueous or hydro alcoholic liquid in a network of colloidal solid particles, which may consist of organic substances, such as polymers of natural or synthetic origin [2]. They have a higher aqueous component that permits greater dissolution of drugs, and also facilitates migration of the drug through a vehicle (that is essentially a liquid) when compared to an ointment or a cream base [3,4]. Gels offer improved potential as a vehicle for topical administration of drugs in comparison to ointment, because they are non-sticky, require low energy during formulation, are stable and have greater aesthetic value. The major advantage of hydrogels is that they are used for simple encapsulation of cells or drugs in homogenous material [5].

Buchananian Lanzan (BL) is a useful tree (family Anacardiaceae) found throughout the hot and dry deciduous forests of India [6]. Different parts of the plant have been used in Indian folk medicine for the management of a variety of diseases. The plant has been effectively used in skin diseases, as a cardio tonic as well as for the management of glandular swelling. This plant is reported to contain flavonols, tannins, glycosides, steroids, saponins and phenols such as gallic acid [7,8,9]. There is a report mentioning the presence of myricetin 3-rhamnoside -3-galactoside in the leaves of BL [10]. In our laboratory, studies with the root extract of BL revealed significant antimicrobial activity against different gram positive and gram negative bacteria. In addition the plant extract was also found to display wound healing (topical) properties, and also displayed anti biofilm properties [11]. A number of biofilm producing microorganisms have been found to delay wound healing and the biofilms have been directly implicated in the resistance to commonly used topical antibiotics [12]. Successful development of a topical hydrogel system requires through investigate of its rheological properties. The objective of the present study was to develop several hydrogel formulations containing the extract of BL and to evaluate their rheological properties for assessing their suitability as topical hydrogel system.

MATERIALS AND METHODS

Materials

Carbopol 940, Methyl paraben, Propyl paraben, Propylene glycol, and Triethanolamine were purchased from Merck Ltd, (India), "MEGAHEAL" Topical hydrogel was purchased from Aristo Pharmaceutical Pvt.

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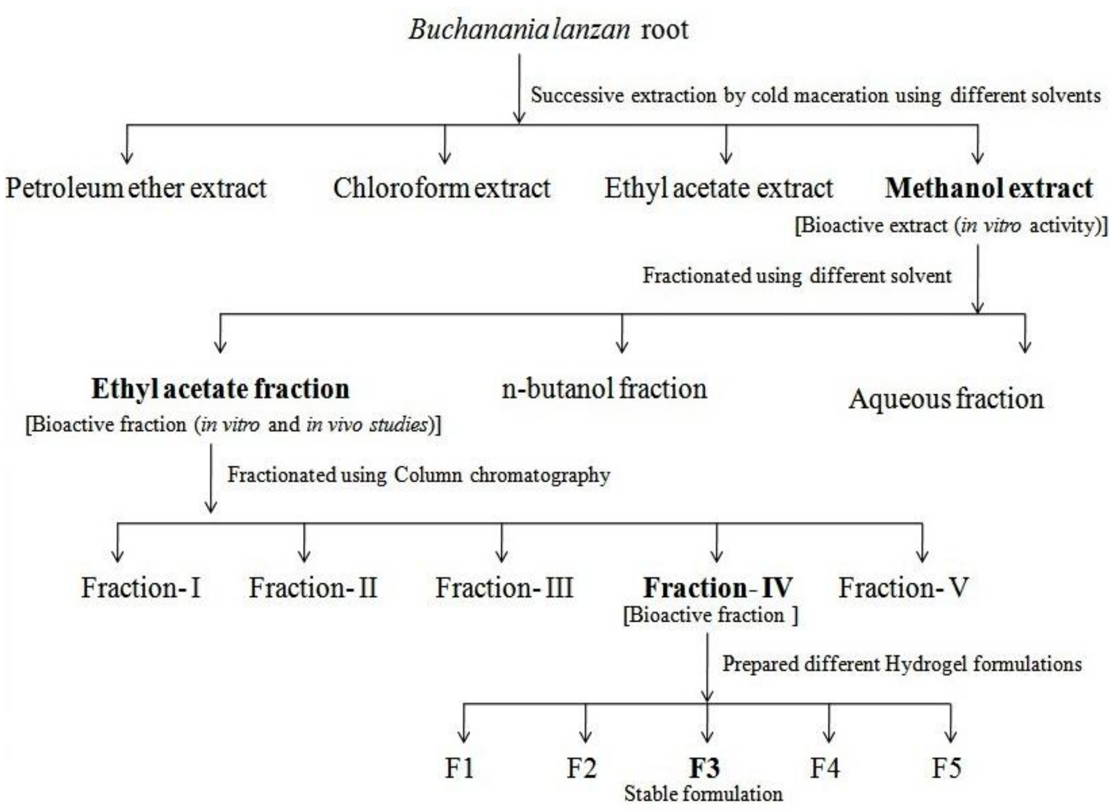

Figure 1: Schematic diagram of bio-guided fractionation of methanolic root extract of *Buchanania lanzan* (MEBL) and preparation of different formulations (F1-F5)

Ltd, (India).The other chemicals were of analytical grade and procured from RANKEM Chemicals,(India).

Collection, Authentication and Preparation of plant sample

The plant was identified and authenticated by the Central National Herbarium, Botanical Survey of India (BSI) P.O.: Botanical Garden, Howrah, India [No.- CNH/I-I (81)/2005-Tech.II./1134]. The plant specimen (Herbarium) was also deposited to the Department of Pharmaceutical Sciences, BIT, Mesra, Ranchi, India.

The roots of BL were dried in shade for about a week followed by drying at 38ºC in an oven for a day. The roots were then ground to a coarse powder and the resulting powder was passed through a sieve (20 BS). Finally, this powder was used for extraction.

Preparation of extract and activity guided fractionation

Successive extraction of powdered plant materials were done by cold maceration using petroleum ether, chloroform, ethyl acetate and methanol as solvents [13]. In this method plant materials (500 gm) were continuously macerated in an air tight, clean flat bottomed glass container for 7 days at room temperature with occasional stirring and shaking. The extract obtained was decanted and clarified by filtration and was concentrated in a rotary evaporator (Buchi Laboratechnik-AG, Switzerland) [14].

All the fractions were kept in desiccators for future use. The MEBL was concentrated in rotary evaporator (Buchi Laboratechnik-AG, Switzerland) and kept at 4ºC until further use. The methanol extract was further subjected to activity guided fractionation using different solvent systems [15] as demonstrated in Fig. 1 The fraction labeled as Fraction –IV in Fig. 1 was found to display superior biological activity as compared to the other fractions hence this was further utilized for formulation studies.

Insert Fig. 1

Preparation of Topical hydrogel

The bioactive ethyl acetate fraction (0.5%) of MEBL was then utilized for formulation of hydrogels using different concentrations of polymer (carbopol 940) and the formulation details have been depicted in Table 1.

Insert Table 1

Method for preparation of hydrogel containing active fraction of extract

The hydrogel formulations were prepared using carbopol 940, propylene glycol 400, methylparaben, propyl paraben, tri-ethanolamine and distilled water in a quantity sufficient to prepare 50 g of gel. The water required for these formulations was divided into two parts. In one part, the exact amount of Fraction –IV was dissolved and the calculated quantity of propylene glycol-400 and ethanol were added to this solution, carbopol-940 was dissolved and to the other part followed by the addition of methylparaben, propylparaben . Both of these solutions were mixed in a beaker and triethanolamine was added to the mixture drop wise, to obtain the desired pH (6.8-7.0) and the gel consistency [16,17].

Physical Evaluation

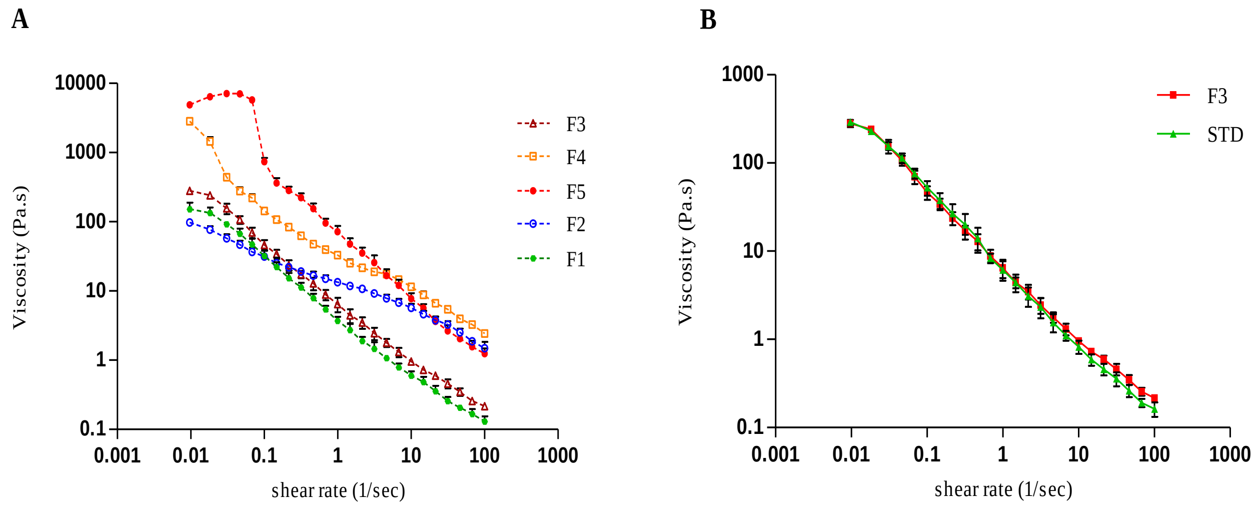

Figure 2: Alteration of viscosity with shear rates has been displayed in the flow curves (A) F1, F2, F3, F4 and F5 and (B) F3 and the standard formulations. The experiments were performed at 25°C

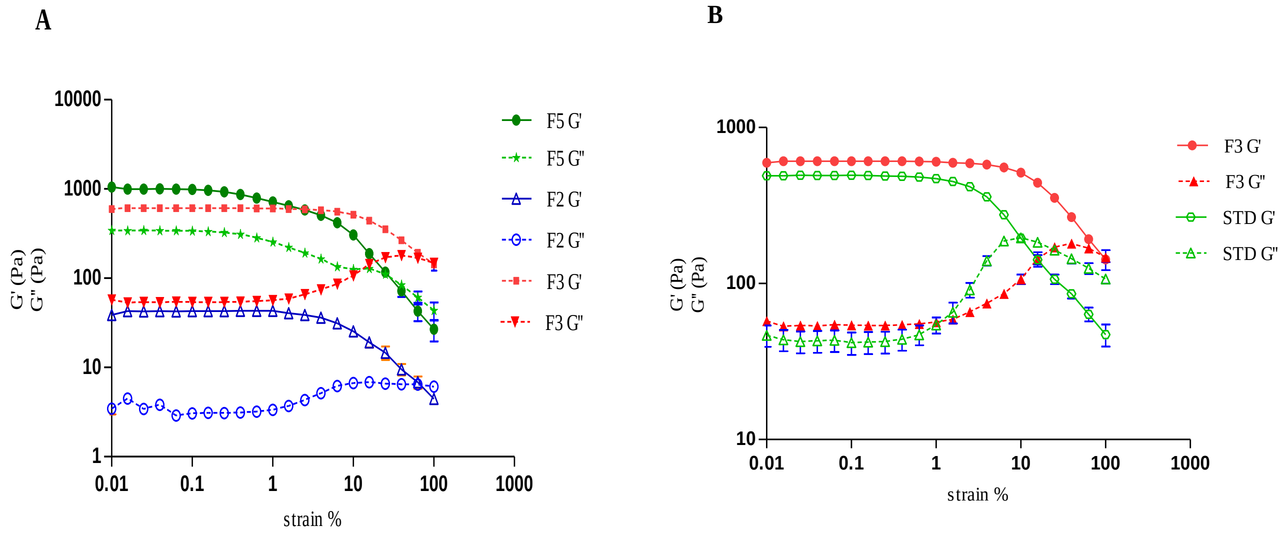

Figure 3: Strain sweep in formulations F2, F3, and F5 (A); F3 and standard formulation (B). The experiments were performed at constant frequency (1Hz) and 25°C

The physical evaluations of the different formulations were performed alongside a commercially available herbal hydrogel formulation (MEGAHEAL).

Appearance and Homogeneity

After the hydrogels have been set in the container were tested for physical appearance, colour and homogeneity by visual observation [18].

Measurement of pH

The pH measurements of the hydrogels were carried out using a digital pH meter (Systronics, India) at 25°C [19].

Spreadability

In order to determine the spreadability of the hydrogels, all formulations and standard reference were taken in between two glass plates (20 cm ×5 cm) each and the time taken for the upper glass plate to slide and separate (when tilted to 45°) was noted [20,21]. The experiment was done in triplicate and spreadability was calculated as follows: $S = M \times L/t$, Where, S = Spreadability, L = Length of the glass plate. W=Weight tied to the upper plate, t = Time taken (second).

Study of Rheological properties

In the present investigation rheological properties were analyzed by (i) rotational and (ii) oscillatory test models. All the rheological parameters were studied using a Modular Compact Rheometer (Model no. MCR 102, ANTON PAAR) . A standard cone geometry (CP-40; 40 mm of outer diameter, angle = 1°) was employed for the test. The rheological parameters like flow curve (viscosity against shear rate), amplitude sweep (storage modulus (G') and loss modulus (G") against % strain), and frequency sweep (G', G" against angular frequency) were studied using Rheoplus software (US 200 version 3.62) [22]. The experiments were performed in triplicate.

Rotational test

One of the major parameter in rotational test is analysis of the flow curve that deals with the dynamic viscosity

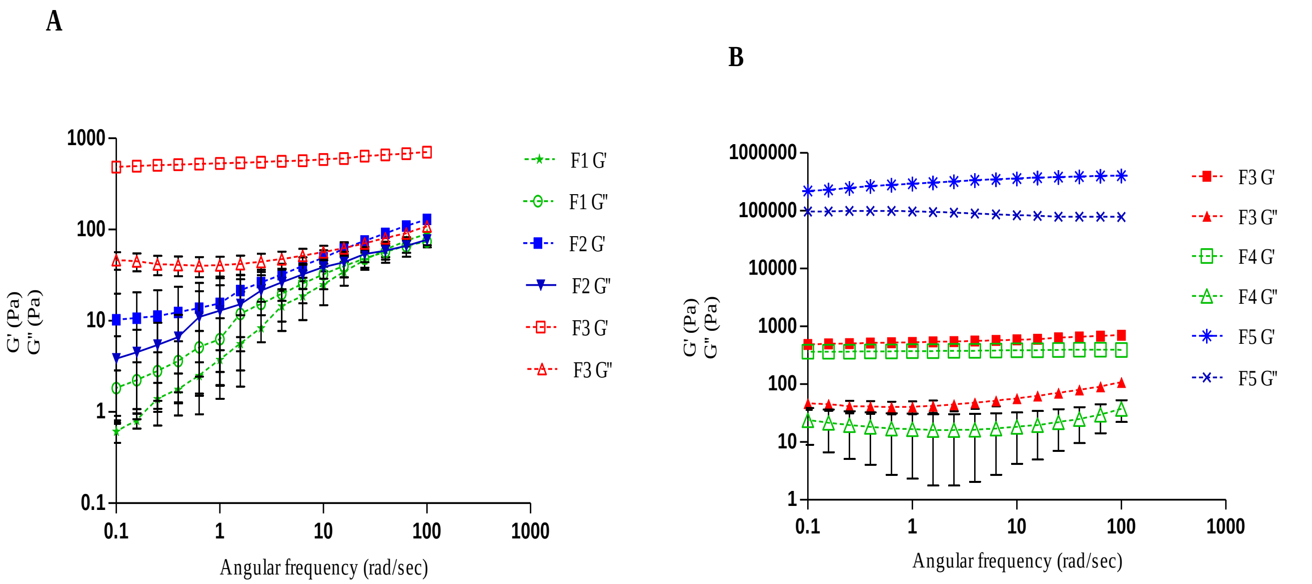

Figure 4: Frequency sweep test was performed with the different formulation at 25°C. (A) F1, F2 and F3 and (B) formulations F3, F4 and F5

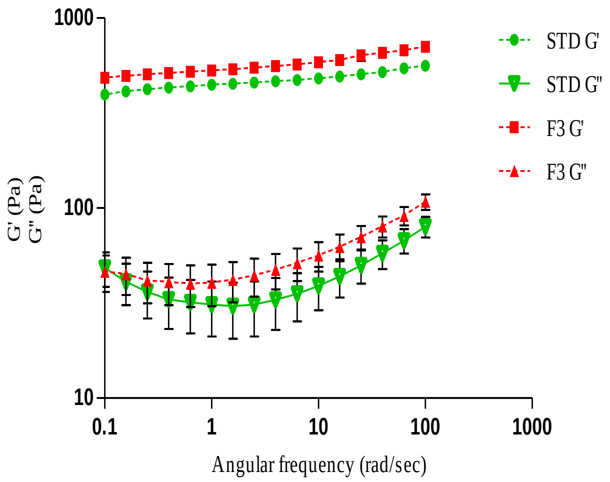

Figure 5: Frequency sweep in formulation F3, and the standard formulation at 25°C

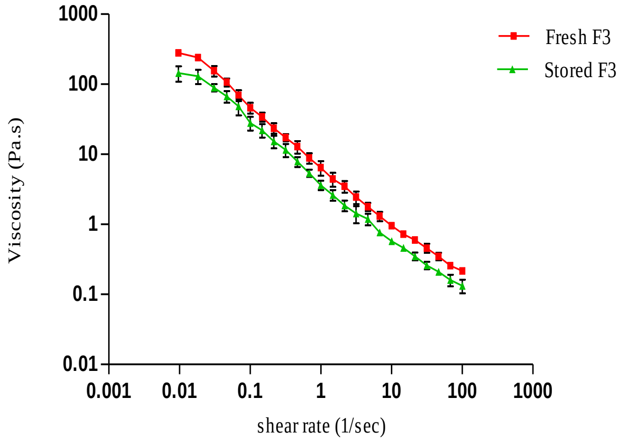

Figure 6: Flow curve of freshly prepared F3 and the stored formulation F3 (40°C ±2°C, 75% RH ± 5% for 3 months). The rheological analysis were performed at 25°C

changes with shear rate. The flow curve of all formulations with the reference standard were determined at 25 °C, for obtaining the changes in viscosity (Pascal second or Pa.S) with different shear rates (1/Sec) [23] for determining the comparative flow behavior of the hydrogels. All plots were taken in logarithmic scale.

Oscillatory test

The oscillatory test for the hydrogels were performed to study the amplitude sweep or strain sweep,[24] and frequency sweep [25]. The amplitude sweep was studied to determine the linear visco elastic region or range (LVE) of the hydrogels. The test was carried out for all the samples at constant temperature (25 °C) and frequency (1 Hz). The strain (%) was varied in order to produce structural deformation of the materials, thereby changing the LVE of the formulations [26].

Frequency sweep

The strain determined from the amplitude sweep test (calculated % strain within LVE) was applied as a constant parameter for the frequency sweep test (carried out at 25 °C), performed under oscillatory mode. The changes in the storage modulus or G' (Pascal or Pa) and loss modulus or G" (Pascal or Pa) against angular frequency (ω) (radian /sec or rad/sec) plots of all the formulations and standard reference were recorded in logarithmic scale that describes the comparative rheological stabilities of the gel structures.

Stability study

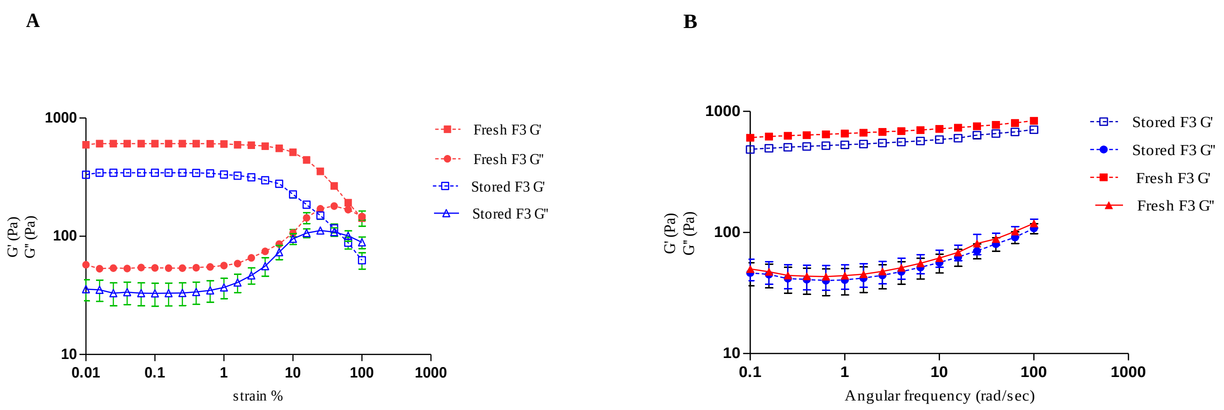

Figure 7: Comparison of amplitude sweep (A) and frequency sweep (B) between the freshly prepared formulation (F3) and the stored (40°C ±2°C, 75% RH ± 5% for 3 months) F3. The rheological analysis were performed at 25°C

Table 1. Composition of the gel formulations prepared with methanolic root extract of *Buchanania lanzan* (MEBL)

Formulations	Plant extract (mg)	Carbopol 940 (mg)	Methyl paraben (mg)	Propyl paraben (mg)	Propylene glycol (ml)	Triethanolamine (ml)	Purified water (ml)
F1	250	300 (0.3%)	25	5	10	Up to 2.5	Q.S up to 50
F2	250	400 (0.4%)	25	5	10	Up to 2.5	Q.S up to 50
F3	250	500 (0.5%)	25	5	10	Up to 2.5	Q.S up to 50
F4	250	600 (0.6%)	25	5	10	Up to 2.5	Q.S up to 50
F5	250	800 (0.8%)	25	5	10	Up to 2.5	Q.S up to 50

Table 2. Composition of the gel formulations prepared with methanolic root extract of *Buchanania lanzan* (MEBL)

Formulations	Color	Appearance	Mean Spreadability (g.cm/s)	±SD	Mean pH	±SD
F1	Light brown	Clear and Transparent	52.66	+0.15	6.85	+0.03
F2	Light brown	Clear and Transparent	46.80	+0.02	6.92	+0.02
F3	Deep brown	Clear and Transparent	35.24	+0.04	7.07	+0.06
F4	Light brown	Clear and Transparent	32.33	+0.04	6.91	+0.01
F5	Light brown	Clear and Transparent	29.79	+0.04	7.21	+0.02
Standard	White	Clear and Transparent	31.49	+0.06	7.14	+0.04

The stability study was performed as per ICH guidelines for the optimized formulation (on the basis of physical characterizations) was subjected to accelerated stability studies at (40°C ± 2°C, 75% RH ± 5%) for 3 months [27]. The formulations were observed for possible changes in colour, odour, consistency, pH, rheological properties and spreadability [28].

RESULTS AND DISCUSSION

Physical Evaluation

The formulations were prepared with different concentration of carbopol and the physical characteristics of various gels are shown in Table 2. From the results, it is clearly evident that all the gel formulations showed good gelling property and homogeneity. The different gel formulations were of light brown colour and were transparent in appearance. The formulation labeled F3 demonstrated a smooth feel on application and the same property was retained even after the end of 3 months. The pH of the formulation F3 was also found to be similar to that of the freshly prepared F3. The rheological behaviors of the gel formulations were also studied. The gel formulation F3 displayed better rheological stability, flow behavior, improved stability and spreadability as compared to the others. However the standard formulation displayed a lower spreadability (31.5 g.cm/s) as compared to F3.

Insert Table 2

Study of Rheological parameters

Flow curve

In rotational methods the test material is continuously sheared between two surfaces, one or both of which are rotating. In general, rotational methods are better suited for the measurement of concentrated suspensions, gels and pastes [29]. These devices have the advantage of being able to shear the sample for an unlimited period of time, thereby enabling the monitoring of transient behavior or an equilibrium state to be achieved, under controlled rheometric conditions. In this study it was noted that the viscosities of all hydrogels decreased with increasing rate of shear, moreover, increase in the concentration of carbopol (F5, F4) led to an increase in viscosity as shown in Fig. 2A The F3 gel was found to possess rheological properties (flow pattern) similar to that of the standard reference gel (Fig. 2B).

Insert Fig. 2A and Fig. 2B

Amplitude sweep or Strain sweep

Forced harmonic oscillation is a dynamic rheometric test in which both stress and strain vary harmonically with time, and both viscous and elastic parameters are derived from the material response. Such tests are almost always carried out in the linear visco-elastic region, which is characterized by a linear response of dynamic viscosity and elasticity with increasing strain amplitude [30].

The amplitude sweep has been used for differentiating between weak and strong gels and this property provides information about the structural strength of the material. Strong gels may remain in the LVE region withstanding greater strain than weak gels and such strong gels have been found to possess linear visco-elastic behavior in higher strains.[31] Amplitude sweep was performed to determine the LVE region (average % strain) of the particular material and this value was used as a parameter in the frequency sweep experiment.

After analyzing all the hydrogels, it was found that the gel containing lower concentration of carbopol (F2,) displayed lower G', G" values with short LVE as compared to (F3, F5) with different LVE range (Fig. 3A). Material with long LVE can possesses more resistance against structural deformation due to oscillatory stress [32]. In this study, the G' of F2, F3 and F5 were found to be greater than G" shown in Fig. 3A, thereby indicating the formation of elastic gel like structure [33]. From Fig. 3A it is evident that F5 displays significantly higher LVE and G' values indicating the formation of strong elastic gel networks that may affect both spreadability as well as permeability. However, F3 displays a moderate LVE range when compared to the other formulations including the standard hydrogel as shown in Fig. 3A, and Fig. 3B. Hence, F3 may be considered as a rheologically stable gel formulation as compared to the other formulations.

Insert Fig. 3A and Fig. 3B

Frequency sweep

According to reports, the G′ of visco-elastic liquid is known to be lower than G″, whereas in visco-elastic solids, G' is found to be higher than that of G″. If G′ crosses G″ then it is referred as a crossover point (where G′ = G″), it is also considered as mechanical stress of material. If a material does not show any crossover point then it is considered that the material is a stable visco-elastic solid (when G′ > G″) or visco-elastic liquid (when G″ > G′), with very high mechanical strength. It may also be mentioned that material with very higher mechanical strength results in higher yield stress that is known to affect permeability [34]. From the frequency sweep test it was found that all samples including the standard, displayed higher G′ values than G″. Among all the samples only F1 showed the crossover point (Fig. 4A) thereby indicating the instability of the formulation, whereas F2 did not display the crossover point but had a small difference between G′ and G″ (within the LVE range) indicating that the higher concentration of Carbopol leads to a very stable and strong gel like structure [35], as was also observed in both F4 and F5 when compared to that of F3 in Fig. 4B. From the Fig. 4A and Fig. 4B it was also noted that values of G' for the different formulations follow an order that may be represented as F5>> F3>F4>F2>F1. Thus from our findings, F3 was found to display a moderately strong gel-like structure similar to that of the standard hydrogel (Fig. 5).

Insert Fig. 4A, Fig. 4B and Fig. 5

Stability study

Based on the all physical studies performed with the different formulations (in Table 1), F3 was found to be stable, hence this formulation was further subjected to accelerated stability studies (at 40 °C ±2 °C, 75% RH ± 5% for 3 months). After 3 months of storage F3 did not display any significant change in colour, pH, rheological properties and spreadability. In addition, the aged sample of formulation F3, when compared to a freshly prepared formulation F3, displayed negligible changes in flow curve, amplitude sweep and frequency sweep as shown in Fig. 6, Fig. 7A, and 7B, respectively.

Insert Fig. 6, Fig. 7A and Fig. 7B

CONCLUSION

In the present study the hydrogel formulations of MEBL fractions were prepared using Carbopol-940. The prepared herbal hydrogels were evaluated for pH, rheological characteristics, spreadability and stability studies. From the physical evaluations it was observed that all the gel formulations displayed good gelling property, homogeneity, and rheological properties. Among the different formulations, F3 was found to be optimum in terms of stability, consistency, spreadability and rheological behavior. On the basis of these observations, the formulation F3 was found to be the most promising. The optimized formulation F3 demonstrates all the characteristic features of a good gel formulation when compared to that of a commercially available hydrogel. Moreover, further studies are in progress to evaluate and characterize the formulations using various physico-chemicals and in vivo experimental models.

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