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Original Research Article

Carbopol hydrogel/sorbitan monostearate-almond oil based organogel biphasic formulations: Preparation and characterization of the bigels

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Abstract

Purpose: *To obtain and evaluate carbopol hydrogel/sorbitan monostearate-almond oil-based organogel biphasic formulations (bigels) as a semi-solid vehicle for medicated topical applications.*
Methods: *Bigel formulations were obtained under mild conditions at a hydrogel/organogel ratio of 80/20, 70/30, and 60/40 (w/w). Their stability, viscosity, spreadability, microarchitecture, and acute skin toxicity were evaluated.*
Results: *Two formulations, prepared at ratios of 80/20 and 70/30, were stable based on intermediate stability testing, and had a similar viscosity and spreadability (38.0 ± 1.0 mm and 37.3 ± 0.6 mm, $p > 0.05$, respectively). Both of these formulations had a bimodal droplet size distribution and very similar values for the droplet mean diameter (0.33 ± 0.05 µm and 2.35 ± 0.44; and 0.34 ± 0.04 µm and 2.59 ± 0.21 µm). The formulation obtained at a ratio of 60/40 was unstable during storage. The in vivo results did not reveal any signs of skin toxicity.*
Conclusion: *Considering their beneficial properties, the developed bigels are a potential semi-solid vehicle for topical application and exhibit a moisturizing effect.*

Keywords: *Almond oil, Bigels, Carbopol hydrogel, Moisturizing effect, Organogel, Sorbitan monostearate*

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INTRODUCTION

Bigels are innovative formulations that constitute two-phase structured systems obtained by mixing hydrogel and organogel. These systems can overcome the disadvantages of both types of gels, including the limited ability to cross lipophylic barriers of the skin for hydrogels and the low patient compliance for organogels due to their stickiness and oily residues [1]. They can also combine all advantages of both drug formulations: (i) the ability to accommodate both hydrophilic and lipophilic drugs; (ii) ensure topical or transdermal drug delivery; (iii) provide controlled drug delivery; and (iv) improve patient compliance [1,2]. Furthermore, Almeida *et al* [3] demonstrated that bigel formulations have an enhanced moisturizing effect. These properties make them very promising candidates for topical formulations.

Olive oil [2], sesame oil [4,5], sunflower oil [6],

soybean oil [1,7] and fish oil [8,9] are the most commonly used oils for bigel preparation. Oleogels prepared from these oils are combined with hydrogels of natural and synthetic polymers: guar gum [4], gelatine [1], sodium alginate, hydroxypropyl methylcellulose [8], and carbopol [5,9].

Almond oil has several advantageous properties [10,11] and is commonly used in cosmetic products. Sorbitan monostearate (Span® 60, SMS, hydrophilic-lipophilic balance value 4.7) is a hydrophobic nonionic surfactant used in pharmaceutical formulations as a water-in-oil emulsifying agent. This compound gels several organic solvents, as well as a range of vegetable oils [4,5,12]. The aim of this study was to obtain and evaluate carbopol hydrogel/SMS-almond oil based organogel biphasic formulations (bigels) as a vehicle for semi-solid drug formulations with a topical application.

EXPERIMENTAL

Materials

All materials used in the study including Carbopol® 940 (The Lubrizol Corporation, USA), propylene glycol (Sigma-Aldrich, USA), Span® 60 (Sigma-Aldrich, USA), 95 % ethanol (v/v), almond oil, triethanolamine, and purified water were of pharmaceutical grade.

Animals

The *in vivo* experiment was approved by the Bulgarian Food Safety Agency (permit no. 88/09.01.2014) and Ethics Committee of the Medical University of Plovdiv, Bulgaria (approval no. 1/22.01.2015) [13]. The experiment was performed in compliance with the Basel Declaration [14] and ICLAS Ethical Guideline for Researchers [15].

Six male Wistar rats (weight of 180 – 400 g) were used and maintained under standard laboratory conditions (temperature 22 ± 1 °C, humidity 45 %, and 12-h light-cycle). The rodents received food and water *ad libitum* [13].

Bigel preparation

To prepare bigels, both phases (hydrophilic and lipophilic) were obtained separately. The hydrogel contained Carbopol® 940 1.0 % (w/w), propylene glycol 5.0 % (w/w) [16], ethanol 95 % (v/v) 10.0 % (w/w), and purified water 84.0 % (w/w). The organogel contained almond oil 85.0 % (w/w) and Span® 60 15.0 % (w/w). Briefly, the preparation was prepared as follows: the weighted Carbopol® 940 was dispersed in the mixture of purified water, propylene glycol, and ethanol (25 °C, 400 rpm). A stable hydrogel was formed after adding triethanolamine (pH 5.5 – 6.5). Span® 60 was dissolved in almond oil (60 °C, 100 rpm). A bigel was obtained when the heated organogel was added to the hydrogel under continuous stirring (500 rpm) to obtain a homogeneous mixture and with cooling to ambient temperatures. Three bigel formulations were prepared in hydrogel/organogel ratios of 80/20, 70/30, and 60/40 (w/w); these were named BG20, BG30, and BG40, respectively.

The prepared bigels were inspected visually for their color, homogeneity, consistency, and phase separation [7].

Optical microscopy

An optical microscope (Leica DM2000 LED, Leica Microsystems, Germany) equipped with a camera (Leica DMC 2900) and software for image processing (Leica Application Suite, LAS) was used. Samples were observed at a magnification of 40x after staining with a solution of methylene blue (methylthioninium chloride) 1.5 % (w/v).

Stability study of the bigel formulations

Intermediate stability studies of the three bigel formulations were prepared according to the ICH guidelines. Bigels were packed in tightly closed plastic containers and stored at 30 ± 2 °C/65 ± 5 % RH for 6 months [17]. Their physical stability was evaluated through observation for any change in color, phase separation, or syneresis at regular time intervals [5,18]. To assess changes in their microarchitecture during storage, they were subjected to optical microscopy, as described above.

Droplet size distribution

Droplet size distribution analysis of the tested formulations was performed using a Nanotrac Wave apparatus (Microtrac, Inc., USA). Microtrac software automatically provided information on multi-modal distributions. The samples were prepared using an equal quantity of bigels (1 % w/w) in phosphate buffer at pH 5.5. They were subjected to ultrasonic impact for 10 min to obtain a homogeneous emulsion before measuring the droplet mean diameter. Samples with a volume of 3 mL were analyzed at 25.0 ± 0.5 °C. The measurements were performed three times and the results were recorded as the mean ± standard deviation (SD) [5].

Determination of pH

The pH values of 1 % aqueous dispersion of the prepared bigels were measured using a pH meter (inoLab pH 720), which was calibrated with standard buffer solutions. The measurement was performed three times and the pH of every bigel formulation was recorded as the mean value ± SD.

Determination of viscosity

Determination of the viscosity of bigel formulations was performed using a Selecta STS-2011 viscometer (J. P. SELECTA, s. a., Spain) at 25.0 ± 0.5 °C. A spindle R6 was used at 1, 2, 3, 4, 5, 6, and 10 rpm. The results were plotted after triplicate measurements (mean ± SD).

Determination of the spreadability of bigel formulations

Spreadability of the bigel formulations was evaluated as follows: 1 ± 0.01 g of sample was pressed between two horizontal plates (20 x 20 cm), where the upper plate weighed 125 ± 1 g. The study temperature was maintained at 25.0 ± 0.5 °C and the bigels were evaluated 48 h after their preparation [19]. The spread diameter (ϕ) was measured after 1 min [19-21]. The measurements were performed three times and the results were recorded as the mean ± SD [5].

ATR-FTIR analysis

ATR-FTIR analysis was performed with a NicoletTM iS TM 10 FT-IR Spectrometer equipped with a Smart iTRTM attenuated total reflectance sampling (ZnSe crystal) accessory (Thermo Scientific, Thermo Fisher Scientific, Inc., USA). The spectra were recorded from 4000 to 650 cm^{-1} using a DTGS detector. All spectra were corrected for CO_2 using internal software.

Acute skin toxicity testing

Six rats (weight 250 – 400 g) were selected for the skin toxicity tests (n = 6). Animals were anesthetized with an intraperitoneal injection of ketamine (90 mg/kgbw) and xylazine (10 mg/kgbw). Fur on the back of the rats was shaved and two fields with an area of 1 cm^2 were marked. The sites were chosen to be as close to the rat neck as possible to avoid ingestion through animal licking of the site after treatment. One field was left untreated and one milliliter of the tested formulation was rubbed gently on the skin at the other site. The animals were returned to their cages and observed for symptoms of local and systemic toxicity. Skin toxicity was evaluated on the 1st, 24th, and 48th hour after application. The results were photographed and irritation scores were determined using a modified Draize scoring system [22].

Statistical analysis

Statistical analysis was performed using a one-way ANOVA with GraphPad InStat software 3.10 version [23]; $p < 0.05$ was considered to indicate significance.

RESULTS

Characteristics of bigel formulations

The gel formation was confirmed based on the tube inversion test [5] (Figure 1). BG40 had a different structure and consistency from that of BG20 and BG30, possibly due to the greater amount of almond oil organogel and the technological conditions (temperature, stirring speed, stirring time, etc.) used in its preparation. All bigel formulations had a white color with a creamy appearance and a pleasant scent of almond oil. BG20 and BG30 were not greasy to the touch, unlike BG40, in which the oil droplets could be felt, although there was no phase separation.

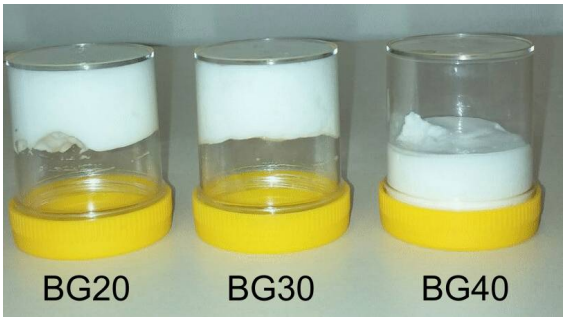

Figure 1: Bigel formulations obtained after mixing of carbopol hydrogel and almond oil organogel

Optical properties

Figure 2 presents optical micrographs of the three bigel formulations in terms of the mutual disposition of the phases. In BG20 and BG30 (Figure 2a and b), the almond oil organogel is dispersed in the form of small gel droplets with regular or oval shapes into the carbopol hydrogel. The oval shape is due to spreading of the bigel formulation on the slide prior to microscopy analysis. Both micrographs show individual droplets with dimensions larger than 5

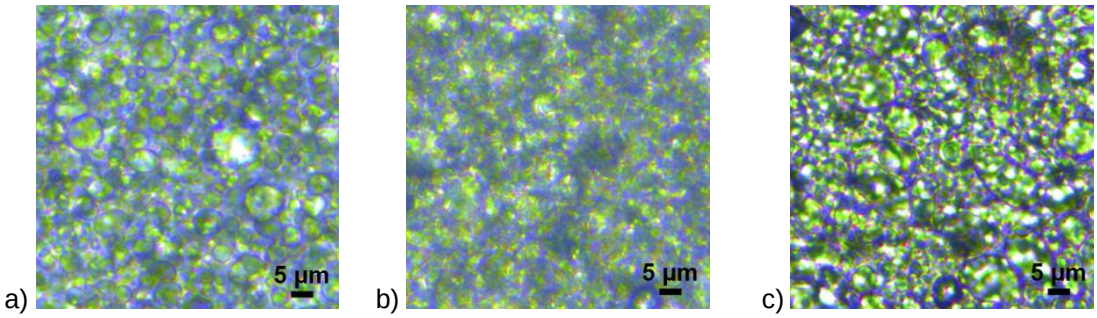

Figure 2: Optical microscopy of bigels at 40x: a) BG20; b) BG30, c) BG40 (Leica DMC2900)

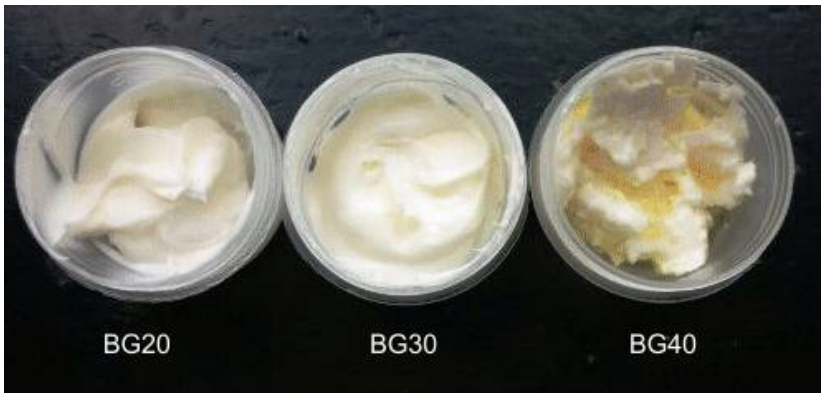

Figure 3: Three bigel formulations after 6 months of storage at 30 ± 2 °C and 65 % ± 5 % humidity

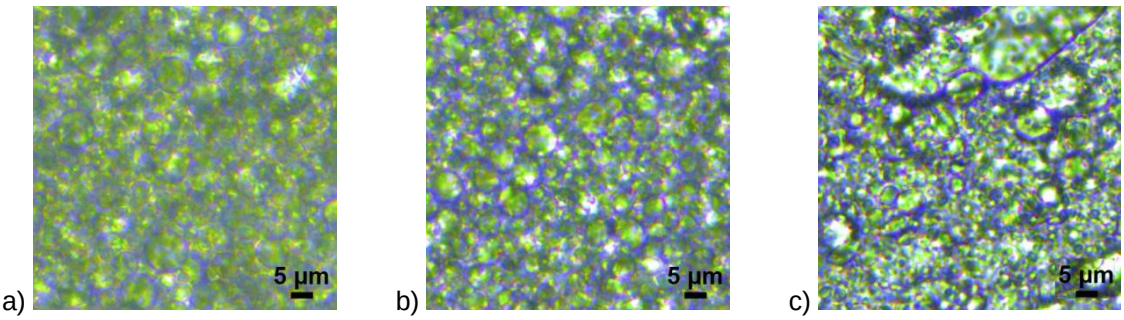

Figure 4: Optical microscopy of bigels at 40x after 6 months of storage at 30 ± 2 °C and 65 % ± 5 % humidity: a) BG20; b) BG30, c) BG40 (Leica DMC2900)

µm. This microstructure of bigel formulations can provide more stability during storage. In contrast, BG40 is characterized by a non-uniform structure due to the presence of large quantities of poorly dispersed almond oil organogel, which is not homogeneously mixed with carbopol hydrogel.\

Stability study of the bigel formulations

Figure 3 shows three bigel formulations after 6 months of storage at 30 ± 2 °C/65 % ± 5 % RH [17]. No changes were observed in the physical stability of BG20 and BG30, as opposed to BG40, during the stability study. Initial changes regarding the phase separation of BG40 were observed early in the experiment after about 1 month storage, after which syneresis occurred.

Optical microscopy was performed on the three bigel formulations to examine changes in their microarchitecture during storage. Figure 4 presents the optical micrographs of BG20, BG30, and BG40 at the end of the intermediate stability study. BG20 and BG30 are characterized by a homogeneous distribution without any areas of confluence of almond oil organogel or carbopol hydrogel. This microstructure plays a crucial role in the physical stability of both bigels (BG20 and BG30). Figure 4c shows the microstructure of BG40, which is characterized by a fragmented and inhomogeneous structure and large, clearly delineated vacuoles indicative of syneresis. Due to the physical instability of BG40, subsequent investigations were performed using only bigel formulations of BG20 and BG30.

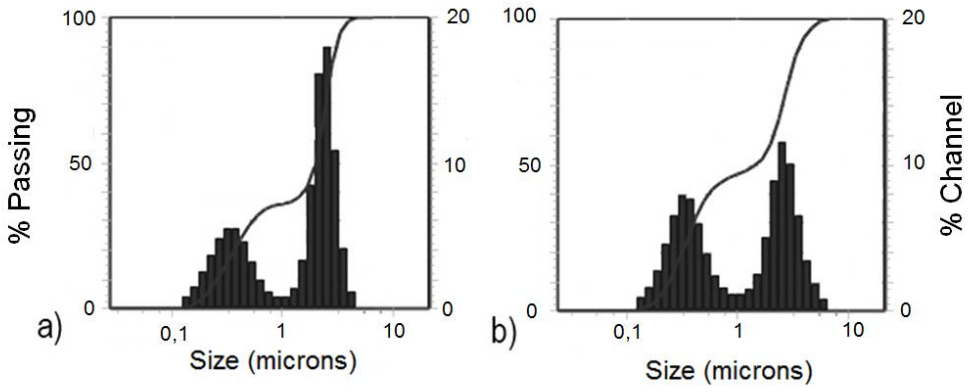

Figure 5: Droplet size distribution of the bigel formulations: a) BG20 and b) BG30

Droplet size distribution

This analysis revealed a broad bimodal droplet size distribution for both BG20 and BG30 (Figure 5). In formulation BG20, 90 % of droplets were larger than 0.257 ± 0.06 µm, and only 5% were larger than 3.29 ± 0.51 µm. Similar results were observed for BG30. The droplet size ranged from 0.244 ± 0.04 to 4.06 ± 0.62 µm. Table 1 shows the peak summary for the bimodal droplet size distribution of the tested formulations. The results indicated that the droplet mean diameter (the 50 % point of each mode reported) was similar in both formulations. A difference in the volume % (the calculated contribution in percent of each peak to the total volume of the distribution) and width (a measure of the broadness of the peak under consideration) was established. Furthermore, the droplet mean diameter established for BG20 and BG30 was in agreement with the results of the optical microscopy analysis, as presented above.

pH of bigel formulations

The pH values of the bigel formulations were 5.66 ± 0.03 and 6.33 ± 0.02 for BG20 and BG30, respectively. These values, which are within the range of the physiological pH of healthy skin, are important for physiological tolerance and a possible lack of skin irritation in topical applications [24].

Viscosity of bigel formulations

Figure 6 shows the effect of shearing on the viscosity of bigels BG20 and BG30. Both bigel formulations exhibited pseudoplastic flow. A lower viscosity of BG20 was established compared to the viscosity of BG30 at all shearing values. An increase in shearing rate led to a decreased difference between the viscosities of both bigels. For example, the viscosities of bigel formulations were ~ 524.7 ± 66 Pa.s and 613.5 ± 52 Pa.s for BG20 and BG30 at 1 rpm; 122.7 ± 17 Pa.s and 150.7 ± 12 Pa.s for BG20 and BG30 at 5 rpm; and 67.1 ± 9 Pa.s and 84.1 ± 5 Pa.s for BG20 and BG30, respectively, at 10 rpm. Statistical processing of the results suggest that the difference is not statistically significant ($p > 0.05$). The higher viscosity of BG30 compared to BG20 can be explained by the higher SMS content in BG30. These results confirm the findings established by Ibrachim *et al* [7].

Determination of the spreadability of bigel formulations

Spreadability is an important characteristic of semi-solid dosage forms: it determines extrudability from the package, the method of application (easy or difficult), accurate dosing, and patient preference. In this study, the spreadability of bigel formulations was determined by measuring the spread diameter (ϕ).

Table 1: Peaks of bimodal droplet size distribution of formulations for BG20 and BG30 (n = 3)

Peak	BG20			BG30		
	Diameter (µm)	***Volume (%)***	***Width***	***Diameter (µm)***	***Volume (%)***	***Width***
Peak 1	2.35±0.44	64.2±7.3	1.15±0.50	2.59±0.21	53.4±5.5	1.76±0.34
Peak 2	0.33±0.05	35.8±7.3	0.30±0.06	0.34±0.04	46.6±5.5	0.29±0.07

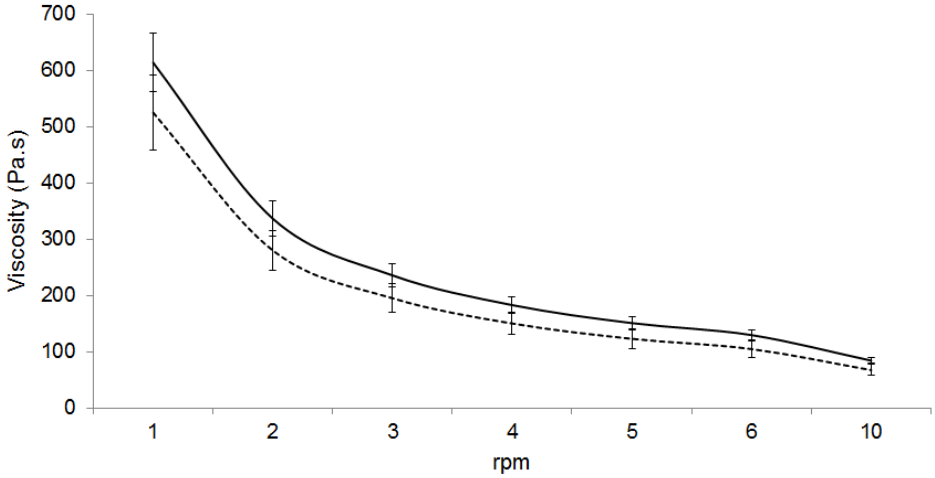

Figure 6: Effect of shearing on the viscosity of bigel formulations BG20 and BG30. Note: ····· = BG20; —— = BG30

The diameters of resulting circles (ϕ) were 38.0 ± 1.0 mm and 37.3 ± 0.6 mm for BG20 and BG30, respectively. In a study on various oil-in-water creams and gels, Arvouet-Grand *et al* [20] and later Lardy *et al* [21] introduce the terms 'semi-stiff' and 'semi-fluid' to evaluate the spreadability of creams and gels. According to both classifications, BG20 and BG30 are semi-stiff formulations, because the established (ϕ) was ≤ 50 mm. Based on these results, the difference between the hydrogel/organogel ratio in both bigel formulations affects the spreadability of BG20 and BG30 ($p > 0.05$).

ATR-FTIR spectra

ATR-FTIR analysis was used to test for any chemical or physical interactions between the two phases: almond oil organogel and carbopol hydrogel. Figure 7 presents the spectra of bigel formulations (BG20 and BG30) and those of both gels before mixing (almond oil organogel and carbopol hydrogel).

Almond oil organogel consists of almond oil and SMS. Its characteristic absorption peaks in the range 2850 – 3000 cm^{-1}, which is associated with the (–CH_2–) group, which is strong and present in the spectra of both SMS [25] and almond oil [26]; the narrow peak located at 1744.51 cm^{-1} is associated with the (=C=O) group [27] and the peak at 1466.21 cm^{-1} belongs to the (–CH_2–) group related to the (–CH_3–) group [27]. In the spectra of both bigel formulations, the peaks at 2921.29 and 2851.58 cm^{-1} were slightly shifted to 2917.86 and 2850.03 and 2917.72 cm^{-1} and 2850.16 cm^{-1} for BG20 and BG30, respectively. They were characterized based on the low intensity, which increased with increasing amounts of almond organogel. The same tendency was observed in terms of the peak localized at 1744.51 cm^{-1} in both spectra of BG20 and BG30.

The carbopol hydrogel consists of Carbopol® 940, propylene glycol, ethanol, purified water, and triethanolamine. This composition is responsible for the following characteristic peaks in the carbopol hydrogel spectrum: the peak located at 3362.03 cm^{-1} is associated with free (–OH) groups; the carbonyl (=C=O) group can be determined between 1670 and 1820 cm^{-1}, but conjugation shifts absorptions to lower wave numbers (the peak at 1644.75 cm^{-1}); and peaks located at 1044.88 cm^{-1} indicate stretching vibration of the ethereal C–O–C group [27,28]. All three peaks are clearly visible in the spectra of bigel formulations of BG20 and BG30.

These results are suggestive of a lack of chemical interaction between the almond oil organogel and carbopol hydrogel, as well as the existence of a physical mixture of the two phases [5].

Acute skin toxicity

The acute skin toxicity test was conducted on six rats with BG30 to reduce the number of experimental animals used and because SMS is present in the bigel. No symptoms of skin toxicity (redness, edema), irritation, or inflammation were observed for the first 48 h after treatment. These results indicate that the tested BG30 formulation can be considered safe for dermal use. Our results are consistent with those of Rehman *et al* [29], who found that bigel formulations did not cause sensitization and may reduce imiquimod (a chemotherapeutic agent) inflammation.

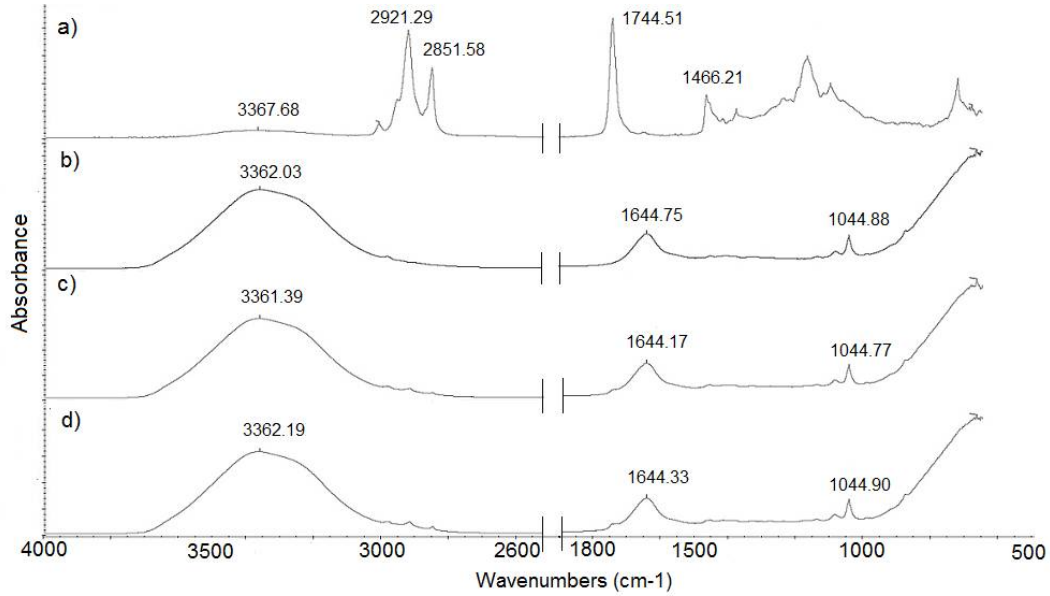

Figure 7: ATR-FTIR spectra of both bigel formulations BG20 and BG30 compared with the spectra of almond oil organogel and carbopol hydrogel: a) almond oil organogel, b) carbopol hydrogel, c) BG20, and d) BG30

DISCUSSION

Three bigel formulations with different carbopol hydrogel/almond oil organogel ratios were developed in the current study. Two formulations, BG20 and BG30, prepared at ratios of 80/20 and 70/30, were stable according to the intermediate stability testing, and they had similar viscosity and spreadability. We observed a bimodal droplet size distribution and similar values for the droplet mean diameter.

Regardless of the broad droplet distribution determined for both bigels, stability was maintained. This may be because most droplets were in the nanometer size range. These results were confirmed based on optical microscopy analysis, as discussed above. Furthermore, BG20 and BG30 constitute a physical mixture of carbopol hydrogel and almond oil organogel (which improves skin tolerance), which was confirmed based on acute skin toxicity testing and in accordance with the pH of the formulations. If a chemical interaction between the components of the bigels was observed, it would have been a prerequisite for skin sensitivity and for a change in the physical and mechanical characteristics of the system, which would affect stability and behavior as a drug delivery system. Both bigel formulations have similar characteristics; they may be used as drug delivery systems for hydrophilic and hydrophobic drugs, and selection of the bigel will depend on the targeted biopharmaceutical characteristics.

Formulation BG40, which contains 40 % (w/w) almond oil organogel, is of particular interest. This system is very unstable and the first signs of physical instability were observed early. Syneresis was observed in the almond oil organogel as part of the bigel composition. The syneresis in BG40 can be explained by the imbalanced interfacial tension between almond oil, SMS, and the gravitational force [30].

In contrast to the results of our study, Singh *et al* [4] found that bigels with a higher proportion of organogel remained stable at high temperatures and maintained their structural organization. According to the authors, the long alkyl hydrocarbon chain of SMS may be the stabilizing factor in this formulation.

Molecules of the gelator tend to self-assemble via nucleation, when the organogelator-solvent melt is supersaturated (*e.g.,* the temperature is below the melting point of the organogelator; 53 °C for SMS). The growth is preferentially one-dimensional, which leads to the formation of fibers. The degree of supersaturation is the primary factor influencing nucleation and growth. The stable state of organogels depends on: (i) hydrogen bonding; (ii) van der Waals forces as transient forces; (iii) and the mechanism of fiber growth, which is influenced by the nature of the liquid portion of the gel [30]. The presence of almond oil used in the current study may explain the difference in the physical stability of BG40 and the bigel obtained by Singh *et al* [4], along with some technological differences in the preparation and storage of bigels. Rogers *et al* [30] found that differences in the physico-mechanical characteristics of the examined organogels can be explained by the fiber growth model, as well as the storage temperature.

However, further studies are required to explore the physical instability of BG40.

CONCLUSION

An effective method for the development of novel carbopol hydrogel/sorbitan monostearate-almond oil based bigels has been developed. The bigel formulations obtained at hydrogel/organogel ratios of 80/20 and 70/30 (w/w) are characterized by a homogeneous microstructure, viscosity and spreadability that are appropriate for semi-solid formulations. The formulations did not show any signs of skin toxicity. Thus, the developed bigels appear to be suitable semi-solid vehicles for topical applications, as well as good skin moisturizers.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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