Journal of Oleo Science
Copyright ©2014 by Japan Oil Chemists' Society
doi : 10.5650/jos.ess14101
J. Oleo Sci. **63**, *(10) 961-970 (2014)*

JOURNAL OF OLEO SCIENCE
Jos

Development and Physical Characterization of Polymer-Fish Oil Bigel (Hydrogel/Oleogel) System as a Transdermal Drug Delivery Vehicle

Khurram Rehman, Mohd Cairul Iqbal Mohd Amin and Mohd Hanif Zulfakar*

Centre for Drug Delivery Research, Faculty of Pharmacy, Universiti Kebangsaan Malaysia

Abstract: Polymer-Fish oil bigel (hydrogel/oleogel colloidal mixture) was developed by using fish oil and natural (sodium alginate) and synthetic (hydroxypropyl methylcellulose) polymer for pharmaceutical purposes. The bigels were closely monitored and thermal, rheological and mechanical properties were compared with the conventional hydrogels for their potential use as an effective transdermal drug delivery vehicle. Stability of the fish oil fatty acids (especially eicosapentanoic acid, EPA and docosahexanoic acid, DHA) was determined by gas chromatography and the drug content (imiquimod) was assessed with liquid chromatography. Furthermore, in vitro permeation study was conducted to determine the capability of the fish oil-bigels as transdermal drug delivery vehicle. The bigels showed pseudoplastic rheological features, with excellent mechanical properties (adhesiveness, peak stress and hardness), which indicated their excellent spreadability for application on the skin. Bigels prepared with mixture of sodium alginate and fish oil (SB1 and SB2), and the bigels prepared with the mixture of hydroxypropyl methylcellulose and fish oil (HB1-HB3) showed high cumulative permeation and drug flux compared to hydrogels. Addition of fish oil proved to be beneficial in increasing the drug permeation and the results were statistically significant ($p < 0.05$, one-way Anova, SPSS 20.0). Thus, it can be concluded that bigel formulations could be used as an effective topical and transdermal drug delivery vehicle for pharmaceutical purposes.

Key words: bigels, hydrogels, oleogels, fish oil, polymer

1 Introduction

Topical gels are unique materials that are rigid and elastic in nature[1], and have a broad range of applications in the cosmetic, medicine, biomaterial, and food industries[2-4]. Hydrogels are most common and well known type of drug delivery system which are widely used for topical and transdermal drug delivery[1, 5, 6]. Hydrogels could be regarded as first choice of drug delivery vehicles among gel vehicle systems due to its ability to increase hydration of stratum corneum, cooling effect, good spreadability and water washable, but when it comes to delivering the drug across the stratum corneum it does lack the skin permeability and has less compatibility with lipophilic drugs[7]. Due to lipophilic compatibility with stratum corneum, edible oils like palm oil, olive oil, and fish oil had been widely used for their role in pharmaceutical and cosmetic products[8-10]. Drug permeation from hydrogels could be improved with the help of these edible oils. Thus a new vehicle system "bigel" was introduced to improve the shortcomings of hydrogels[11]. It is a semi-solid dosage form prepared by intimately mixing two different colloidal species[12] an oleogel and an aqueous gel for topical and transdermal administration of drugs. As oleogels are considered as oily in nature, difficult to remove after application and less patient compliance, therefore bigels were introduced as an alternative gel system that possess the characteristics of both oleogels and aqueous gels, such as cooling effect, increase in hydration of the stratum corneum, good spreadability, and water washable upon application to the skin. In addition, these gels are compatible with many substances, which increases the emollient and moisturizing effect on the skin and improves the penetration of drugs through the skin[11, 13].

So far very little work has been done on bigels in terms of understanding the composition for such formulation. Polymers have been investigated extensively in the advancement of drug delivery technologies[14]. They provide a controlled and predictable release of the drug and capable of carrying hydrophilic and hydrophobic drugs[14]. To evalu-

*Correspondence to: Mohd Hanif Zulfakar, Centre for Drug Delivery Research Faculty of Pharmacy Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300, Kuala Lumpur, Malaysia
E-mail: hanif@pharmacy.ukm.my
Accepted June 19, 2014 (received for review May 23, 2014)
Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online
http://www.jstage.jst.go.jp/browse/jos/ http://mc.manusriptcentral.com/jjocs

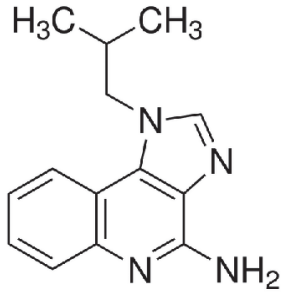

Fig. 1 Structure of imiquimod.

ate the effectiveness of bigels as a potential drug delivery vehicle, bigels from natural (sodium alginate) and synthetic (hydroxylpropyl methylcellulose) polymer along with fish oil were prepared and used as control for bigel formulation. Hydroxypropyl methyl cellulose (HPMC) and sodium alginate are commonly used polymers for the formulation of hydrogels and their capability as a drug delivery vehicle is well known[15-18]. Fish oil was chosen as oil phase in bigels because it contains omega-3 fatty acids (especially eicosapentanoic acid, EPA and docosahexanoic acid, DHA) that have the potential to improve skin permeation and have been associated with therapeutic effects in inflammatory diseases of the skin[19-23].

In this study, bigels of different ratios of hydrogel and oleogel were formulated, without the addition of a surfactant. Imiquimod was selected as a model drug. Imiquimod is considered as a basic drug with molecular weight of 240.30 and log P value of 2.7 and it has a quinolone structure (Fig. 1). Imiquimod is an immune response modifier currently used against skin diseases such as actinic keratosis, basal cell carcinoma, neoplasia, and external genital and perianal warts in adults[24-28]. Bigels were then characterized for their application in topical drug delivery. The aim was to investigate the suitability of fish oil-bigels for the purpose of topical drug delivery vehicle as compared to hydrogel. Thermal gravimetric analysis was also performed to understand the thermal behavior of bigel formulation as compared to hydrogels. Mechanical properties and rheological characterization for spreadability and the permeation studies to determine the capability of the formulations as a drug delivery carrier as compared to hydrogels were also recorded.

2 Materials and Methods

2.1 Materials

All chemicals and materials were of analytical grade and were commercially available. Blackmores Fish Oil (42% EPA and 21% DHA) was purchased from Blackmores (Warriewood, Australia), and benzalkonium chloride, sodium alginate, and hydroxymethyl propyl cellulose were purchased from ACROS (New Jersy, USA). Beeswax was obtained from R & M chemicals (Selangor, Malaysia), cetrimide, butylated hydroxyanisole, and imiquimod powder was purchased from Sigma-Aldrich (St. Louis, USA). Hexane, sodium methoxide, and cellulose acetate membrane filters with a pore size of 0.45 μm were purchased from Sterlitech Corporation (Washington, USA).

2.2 Preparation of the bigel formulation

The scheme for the preparation of bigels is illustrated in Fig. 2a. Bigels were prepared in 3 steps. Initially, the hydrogel was prepared followed by preparation of the oleogel, and both phases were mixed in the last stage to prepare a homogenized gel termed as bigel.

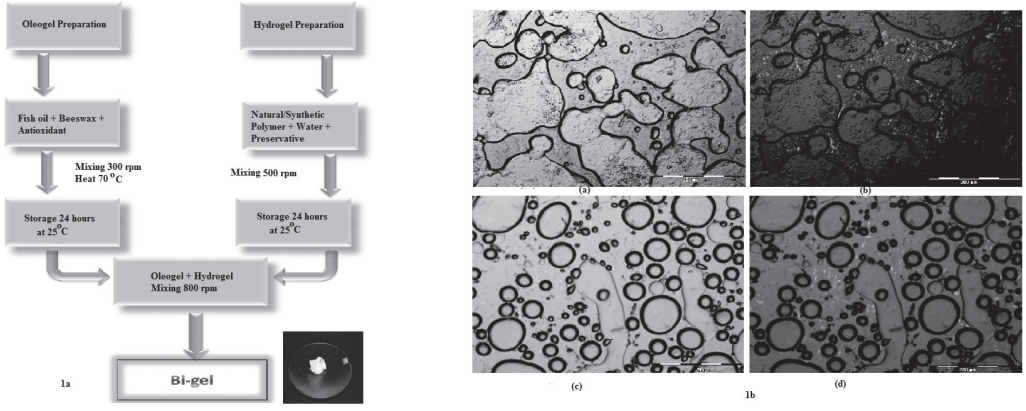

Fig. 2a (a) Schematic diagram of bigel formulation process. **2b**. Hydrogel and oleogel phase distribution inside bigels observed under polarized microscope (a) Sodium alginate bigel exhibiting stained oleogel phase and hydrogel phase. (b). Sodium alginate bigel under polarized lens exhibiting beeswax crystals in oleogel phase. (c) HPMC bigels showing spherical hydrogel phase and stained pink oleogel phase. (d). HPMC bigel under polarized lens with beeswax crystals in oleogel phase.

2.2.1 Formulation of the hydrogel

To prepare the hydrogels, the ingredients were weighed (Table 1a), added, and dispersed under continuous mechanical stirring (500 rpm) for 15 min at room temperature. The hydrogels were stored in an incubator (25℃) for 24 h before being used for the preparation of the bigels. Imiquimod (5%) was only dispersed in control hydrogel formulations (HH1 and SH1).

2.2.2 Formulation of the oleogel

All the oleogel components (Table 1a) were dispersed under continuous magnetic agitation (300 rpm) and heating at 70℃ for 15 min. After solidifying, the oleogel was stored in a glass container and was stored in an incubator (25℃) for 24 h before being used for the preparation of bigels.

2.2.3 Bigel formulation

Bigel formulations were prepared by adding different quantities of the prepared hydrogels to the oleogel at different ratios. The active ingredient (5% imiquimod) was first dispersed in the oleogel, before mixing with the proportion of blank hydrogels (Table 1b). Each bigel was prepared by mixing the corresponding proportion of hydrogel and oleogel by using mechanical stirring (800 rpm) for 10 min at room temperature.

2.3 Optical microscopy

The homogeneity of the formulations was analyzed using a polarized optical microscope (Olympus BX41TF, Tokyo, Japan). Sudan stain (1% w/v liquid paraffin) was used to differentiate the oleogel phase from the hydrogel phase, and to ensure that the formulations were homogenous in nature.

2.4 Stability Studies

All the bigel formulations were placed in tightly closed glass jars and stored at 5℃ in stability chamber (Climacell 707, Germany) and the stability studies were carried out for the period of 6 months. Appearance, pH and concentration of EPA and DHA fatty acids were determined in regular intervals (month 0, 1, 3 and 6) to detect any physical and chemical changes inside the bigel formulations.

2.5 Thermal gravimetric analysis

A Perkin Elmer STA 6000 (Waltham, USA) was used to perform thermogravimetric analysis (TGA) of the fish oil bigel formulations as compared to hydrogels. Approximately 20 mg of formulation was placed in the sample pan for analysis over a temperature range of 50–600℃, at a heating rate of 10℃/min and under a nitrogen purge (25 mL/min). The differential thermogravimetric (DTG) curve was derived from the TGA results using the Pyris 1 software program (Perkin Elmer,Waltham,USA).

2.6 Rheological characterization of bigel formulations

Samples were stored at 25℃ for 24 h before the rheological test. Rheological measurements of bigels were performed using Bohlin Gemini HR nano rheometer (Malvern Instruments Ltd, UK) equipped with a cone and plate measuring system. The experiments were performed using a cone with a diameter of 20 mm with angle 2° angle. The temperature was controlled at 25℃. Viscosity was obtained at 31 different shear rates and evaluation was performed in triplicate. The mean values of the various parameters of 3 samples of each bigel were reported, and the linearity of viscoelastic properties was verified for all samples. Furthermore, thixotrophic characteristics (decrease in viscosity under different shear rates) and shear thinning against time of the bigel formulations was also measured[29].

Table 1a Composition of hydrogel and oleogel formulations.

Ingredients (g)	Oleogel (OG1)	Hydrogel (SH1)	Hydrogel (HH1)
Hydroxypropyl Methylcellulose	–	–	3
Sodium Alginate	–	3	–
Beeswax	10	–	–
Benzalkonium Chloride	–	0.05	0.05
Butylated hydroxyanisole	0.5	–	–
Deionized water	–	100 q.s	100 q.s
Fish Oil	100 q.s	–	

Table 1b Compostion of bigel formulations.

Oleogel: Hydrogel Ratio	SH1-OG1	HH1-OG1
10:90	SB1	HB1
30:70	SB2	HB2
50:50	SB3	HB3

2.7 Texture analysis

Texture analysis was conducted utilizing a Pro CT3 10K (Brookfield Engineering Laboratories, USA) texture analyzer with a 10000 g load cell. A back extrusion test fixture (TA-DEC) was used with a cylindrical probe (diameter 34 mm), and a compression test was performed to determine the mechanical properties (adhesiveness, hardness, peak stress and springiness) of the bigels. Formulations (25 g) were filled into a standard container of base table (TA-BT-KIT) and the probe was compressed until it penetrated 5 mm under the gel surface at a speed of 2 mm/s, and redrawn, inserted, and redrawn again (a 2-cycle test).

2.8 Gas Chromatography (GC) analysis

Fatty acid analysis was conducted using a Gas Chromatography (GC) 2010 instrument (Shimadzu Corp., Kyoto, Japan) with a flame ionization detector (FID). The injector temperature was maintained at 250℃ and the detector temperature was maintained at 275℃. The temperature of the column was maintained at 180℃ for 2 minutes and then gradually increased to 240℃ at a rate of 4℃/min. Nitrogen gas was used as a carrier with a flow rate of 60 cm/s. To determine the quantity of eicosapentanoic acid (EPA, C20:5n3) and docosahexanoic acid (DHA, C22:6n3) in bigels, samples were treated to prepare fatty acids methyl esters (FAMEs). 0.5 mL of 0.5 M sodium methoxide solution (prepared by mixing sodium methoxide powder in anhydrous methanol) was added to a solution of 50 mg of sample in 1 mL of hexane in a 2 ml screw-capped vial. The vial was capped and screwed on tightly. The solution in the vial was homogenized using an Autovortex SA6 (Stuart Scientific, UK) for 1 minute and allowed to stand until the upper layer became clear (5 minutes). The distinct upper layer of methyl ester was separated carefully in a capped vial. Aliquots of 1 μL FAME hexane solution was injected into a highly polar cyanosiloxane column (SP-2380, 30 mm × 0.25 mm × 0.20 μm film thickness) from Supelco (USA).

2.9 Drug assay

Formulations containing 10 mg of imiquimod were analyzed using chromatographic system consisted of an RP-HPLC (Shimadzu LC-20AT with SIL-20A autosampler and SPD-M20A DAD detector, Shimadzu Corp., Kyoto, Japan) and Altima C8 column (4.6 × 150 mm; 5 um). The mobile phase for imiquimod analysis consisted of acetonitrile, water, and phosphoric acid (250:750:10) adjusted to a pH of 2.7, delivered at a flow rate of 1.5 mL/min with an injection volume of 20 μL. The imiquimod was monitored at a wavelength of 254 nm and calculated against a calibration curve.

2.10 In vitro permeation study

An in vitro permeation study was performed using all formulations (50 mg sample) to determine the permeation of drugs through the skin. This permeation study was performed using a Franz diffusion cell (Franz Diffusion Cell, PermearGear Inc., USA), and a cellulose acetate (CA) membrane with a pore size of 0.45 μm and diameter of 25 mm, which was placed between the donor and receptor compartments. The receptor compartment had a volume of 3.5 mL and a contact area of 0.95 cm^2, was filled with cetrimide solution that acted as a receptor medium, maintained at 37℃, and stirred at 300 rpm. Cetrimide solution was chosen as it will be able to act as a solubilising agent for the highly lipophilic fish oil constituents and may also help in simulate the stratum corneum lipophilic environment[19, 30]. 0.5-mL aliquots were withdrawn at different time intervals (1, 2, 4, 8, 16, 24, 32, 40, and 48 h) through the sampling port and replaced with fresh cetrimide solution to keep the volume of the receptor solution constant. Sample imiquimod content was analyzed using an HPLC-UV (Shimadzu LC-20AT, Shimadzu Corp., Kyoto, Japan) at 254 nm (RP-HPLC system and conditions were identical to those described for the drug assay). The cumulative amount of imiquimod that permeated through the surface area (μg/cm^2) of the CA membrane was calculated, and the rate of drug released or flux rate (μg/cm^2/h) for the formulations was determined from the slope of the plot of the cumulative amount of permeated drug using linear regression analysis. The data obtained from the in vitro studies were also plotted using a Higuchi release kinetics model ($Q = K_h t^{1/2}$) as the cumulative percentage of drug release vs. square root of time. Whereas Q is amount of drug released and K_h is the constant reflecting design variables of the system and t is the time in hours.

2.11 Statistical analysis

Statistical significance of in vitro permeation study results was analyzed by using one-way analysis of variance (ANOVA) using SPSS 20 software. A multiple comparison test was used to compare different formulations, and at 95% confidence intervals, p values less than 0.05 were considered to be significant.

3 Results and Discussion

3.1 Optical microscopy

Optical microscopy was conducted to analyze the composition of bigels with the help of a polarized lens and Sudan stain. The oleogel and hydrogel regions inside the bigels were easily be distinguished under the microscope. The hydrogel portion could be spotted as an unstained sphere shaped areas ranging from 25–200 μm in bigels composed of HPMC, whereas in sodium alginate bigels the size ranged from 50-500 μm. Oleogel region inside the bigels appeared as pink-stained regions (Fig. 2b). Rod-shaped bees wax crystals ranging from 10–40 μm were also

observed in stained regions of oleogels using the polarized lens.

3.2 Stability Studies

All the bigel formulations were regularly monitored for their appearance, pH and fatty acid content to detect any physical or chemical changes inside the formulation. The formulations were white to yellowish white in color (Fig. 3). As the concentration of the oil inside the formulations increased they are more yellowish. Generally, all the formulations remained stable at 5℃ with no change in pH (remained between 5.75-6.50) and the fatty acid (EPA and DHA) content was also between 90-110 percent, indicating that the fish oil was protected from oxidation, but there was a slight reduction in fatty acid content during the passage of time (as shown in Fig. 3).

3.3 Thermal gravimetric analysis

To understand the thermal behavior of formulations TGA/DTG was conducted. There were three different thermal events observed in the thermograms of bigel formulations as compared to 2 thermal events of hydrogel formulations (HH1 and SH1). The first two thermal events were same in hydrogel and bigel formulations, dehydration of water around 100℃ and the melting of imiquimod (290–300℃) Another important aspect which was observed in the thermograms of bigels was the melting point (endothermic peak 62-65℃) of beeswax but it did not produce any major impact on the percent weight loss of the bigel formulation because the beeswax appeared to be quite stable and decomposed completely around 400℃. The fourth thermal event was only present in bigels because of the presence of fish oil. The complete decomposition of the fish oil was observed around 450–500℃ (Fig. 4a). As hydrogels are thermo- sensitive, therefore as the temperature

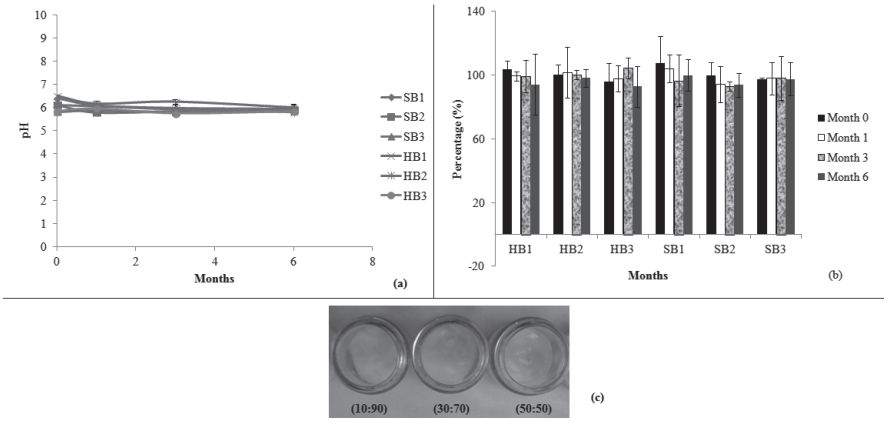

Fig. 3 (a) pH of the bigel formulations over the period of 6 months. (b) EPA/DHA percentage content over the period of 6 months. (c) Appearance of bigel formulations at different fish oil oleogel: polymer hydrogel ratio.

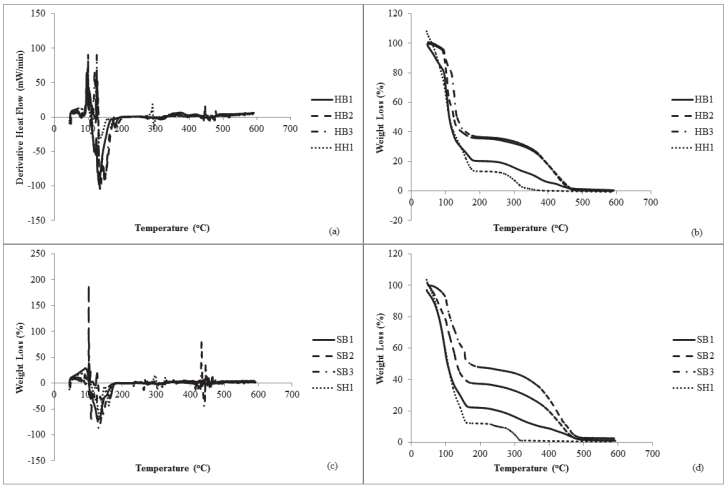

Fig. 4 TGA/DTG Bigel formulation. (a). HPMC hydrogel and bigels TGA/DTG. (b) HPMC hydrogel and bigels percentage weight loss. (c) Sodium alginate hydrogel and bigels TGA/DTG. (d) Sodium alginate and bigels percentage weight loss.

increased, the percentage of weight loss was faster as compared to bigel formulations. Oleogels are known to be more resistant to temperature changes[7, 31], therefore the as the fish oil ratio increased in bigel formulations so does the thermal stability (Fig. 4b). The least weight loss was observed in HB3 and SB3 bigels due to the highest fish oil oleogel ratio.

3.4 Rheological characteristics of the bigel formulations

Rheological behavior for all of the gel formulations was examined. The apparent viscosity (Pa.s) and the shear stress (Pa) of bigel formulations of different composition were measured as a function of shear rate (1/s). The bigel formulations showed non-Newtonian pseudoplastic flow or shear-thinning behavior in which the shear stress versus shear rate curve was convex towards the shear stress axis (Fig. 5). It was observed that hydrogels SH1 and HH1 (1.320 ± 0.025 Pa.s and 1.194 ± 0.154 Pa.s, respectively) had high apparent viscosity and in bigel formulations as the ratio of oleogel increased in the bigels, the apparent viscosity is decreased (Table 2). So in bigels the hydrogel concentration has direct relationship with the rheological features of the formulation. Bigels prepared from sodium alginate hydrogels (SB1-SB3) displayed higher shear stress (Fig. 5) and apparent viscosity as compared to the HPMC-fish oil bigels (HB1-HB3) (Table 2). Increase in viscosity with the increase in ratio of hydrogel in bigel formulations may be because of intermolecular hydrogen bonding in polymer hydrogels, which increases the rigidity of the structure of the bigel formulations. Among bigel formulations with oleogel and hydrogel proportion of 10:90 (SB1 and HB1) had the highest viscosity and shear stress. Viscosity curves under different shear rates and time are also shown (Fig. 6) to further explain the rheological behavior of bigel formulations. The time dependent viscosity changes were observed under different shear rate (as shown in Fig. 6) which indicated thixotropic features of bigels. The non-Newtonian pseudoplastic behavior of bigels contributes to their spreadability when applied on the skin surface. This pseudoplastic behavior can be explained by the flow of the bigels in that at high shear rates, the bigels will flow readily, and in the case of low shear rates, the material will adopt a higher consistency and recover the original rheological properties as those before administration.

3.5 Texture analysis

Texture analysis was performed to analyze the mechanical properties of the bigel formulations and was compared with the properties of hydrogels. It was observed, that the hydrogel concentration contributes into defining the me-

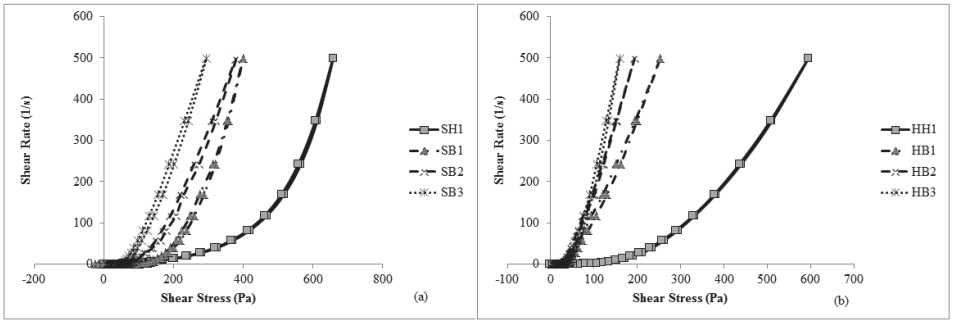

Fig. 5 Rheological features of bigel formulations. (a) shear rate vs. shear stress of Sodium alginate-hydrogel, Sodium alginate-bigels, (b) shear rate vs. shear stress of HPMC hydrogel and bigels.

Table 2 Mechanical properties of hydrogels , and bigel formulations (n=3, Data expressed as mean ± S.D).

Formulation	Viscosity (Pa.s)	Adhesiveness (mJ)	Hardness (N)	Peak Stress (kPa)
SB1	0.920 ± 0.069	6.033 ± 0.642	4.210 ± 0.606	5.956 ± 0.855
SB2	0.845 ± 0.017	3.966 ± 0.862	2.123 ± 0.283	3.005 ± 0.401
SB3	0.589 ± 0.034	3.800 ± 0.360	1.383 ± 0.395	1.956 ± 0.569
SH1	1.320 ± 0.025	7.166 ± 0.833	1.977 ± 0.046	2.797 ± 0.064
HB1	0.508 ± 0.031	14.567 ± 2.983	3.087 ± 0.086	4.362 ± 0.114
HB2	0.388 ± 0.019	6.433 ± 0.503	1.643 ± 0.476	2.326 ± 0.668
HB3	0.319 ± 0.014	4.800 ± 0.871	1.243 ± 0.342	1.757 ± 0.483
HH1	1.194 ± 0.154	19.033 ± 1.457	3.397 ± 0.100	4.809 ± 0.139

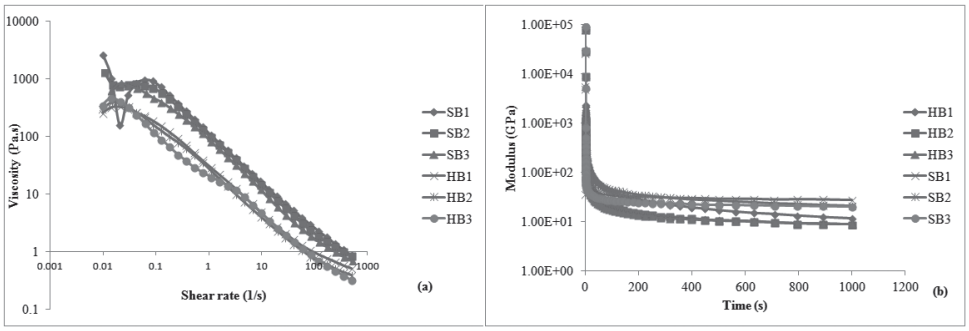

Fig. 6 (a) Viscosity curves of the bigel formulations against shear rate. (b) Complex modulus of the bigel formulations against time.

chanical properties, therefore, hydrogels displayed the highest adhesiveness, hardness, and peak stress values as compared to bigels. HPMC hydrogels HH1 showed high adhesiveness (19.033 ± 1.457 mJ), as did the HPMC bigels (Table 2). Sodium alginate hydrogels SH1 showed lower adhesive values (7.166 ± 0.833 mJ) than HPMC hydrogels, and the Sodium alginate bigels followed the same trend (Table 2).

The firmness of the formulations was determined by hardness and peak stress, which also indicated the strength of the intermolecular forces of the gelling network inside the formulations. Formulations composed of sodium alginate showed greater hardness and peak stress values as compared to the formulations prepared from HPMC (Table 2). The sodium alginate bigel with a 90:10 hydrogel/oleogel ratio (SB1) showed slightly higher peak stress and hardness values than its hydrogel precursor, the hardness and peak stress values decreased, for all the bigels, as the concentrations of hydrogel decreased (Table 2). Thus, hydrogels play a vital role in defining the firmness and intermolecular strength of the formulations, which are due to the hydrogen bonding in the aqueous phase of the formulations.

3.6 Gas Chromatography

Fish oil is composed of omega-3 fatty acids (particularly EPA and DHA). The fatty acid content would indicate the presence of any oxidation, rancidity, or any interaction due to the presence of drug inside the formulation, thus determining the stability of bigel formulations. With help of gas chromatography, fatty acid compositions were determined as fatty acid methyl esters (FAMEs). Fatty acids compositions in fish oil were identified and standardized by comparing the retention time of FAME with the Supelco 37 component FAME mixture standard (Sigma-Aldrich 1996). EPA and DHA inside the bigel formulations were quantified and it was found to be between 91.6-101.4% for all the formulations, which indicated good fatty acid stability (Fig. 7).

3.7 Drug assay

All the formulations were analyzed for imiquimod content by liquid chromatography (Fig. 7). The average drug content for imiquimod was found to be in the range of 90.45–95.77%, which was within the limit (90–110%) provided by the United States Pharmacopeia[32]. This result indicated that the drug added to the formulations during the preparation process was homogenously dispersed and not degraded, and thus the method was reproducible.

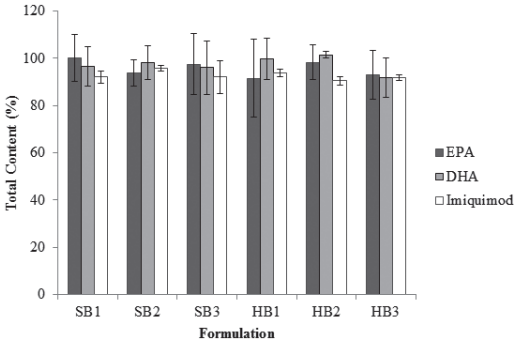

Fig. 7 Content uniformity of imiquimod, EPA and DHA in bigel formulations.

3.8 In vitro permeation study

During in vitro permeation studies sodium alginate hydrogel SH1 showed high permeation (0.6913 ± 0.0154 μg/cm^2) and high drug flux (0.0144 ± 0.0003 μg/cm^2/h) compared to HPMC hydrogel HH1 (0.5648 ± 0.0098 μg/cm^2 and 0.0118 ± 0.0002 μg/cm^2/h). In the Higuchi model equation, the regression value for SH1 was 0.9533, indicating that there was no obstruction in drug release, it was a good fit to the Higuchi model, the release rate of drug from the gel was proportional to the square root of time, and diffusion was the predominant mechanism of drug release. However, for HH1, the regression value of the Higuchi model equation was 0.9166, indicating some obstruction to drug release. The regression value for HPMC bigels (HB1-HB3) was also low compared to the sodium alginate bigels (SB1-SB3) as shown in Table 3. The low regression values may be because of nature of synthetic polymer, as the HPMC is associated with delayed release of drugs from the vehicle

Table 3 Higuchi regression values, cumulative drug release (μg/cm^2), and drug flux (μg/cm^2/h) of hydrogels, and bigels through cellulose acetate (CA) membrane (n=3, Data expressed as mean ± S.D).

Formulation	Higuchi Model (r^2)	Cumulative Drug Permeation (μg/cm^2)	Drug Flux (μg/cm^2/h)
SB1	0.9746	0.9442 ± 0.0219	0.0197 ± 0.0004
SB2	0.9560	0.8726 ± 0.0616	0.0182 ± 0.0013
SB3	0.9454	0.6553 ± 0.0094	0.0137 ± 0.0002
SH1	0.9533	0.6913 ± 0.0154	0.0144 ± 0.0003
HB1	0.9435	0.8072 ± 0.0108	0.0168 ± 0.0002
HB2	0.9590	0.7362 ± 0.0464	0.0153 ± 0.0009
HB3	0.9517	0.7013 ± 0.0600	0.0146 ± 0.0013
HH1	0.9166	0.5648 ± 0.0098	0.0118 ± 0.0002

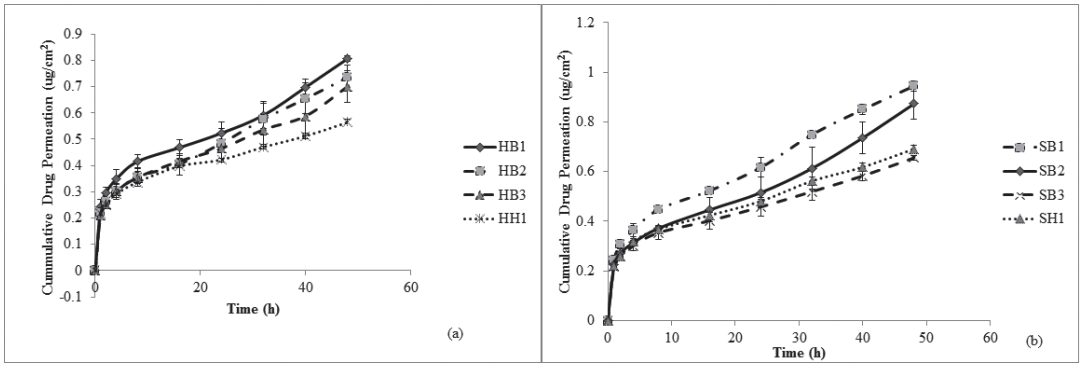

Fig. 8 (a) Cumulative permeation of imiquimod from HPMC hydrogel and bigels. (b) Cumulative permeation of imiquimod from sodium alginate hydrogel and bigels.

due to its cellulose composition. That is why HPMC bigels also displayed lower cumulative drug permeation compared to the sodium alginate bigels (Table 3). For bigels, combination of fish oil oleogel resulted in significant increase ($p < 0.05$, one-way Anova, SPSS 20.0) in the drug permeation as compared to hydrogels. This increase in drug permeation could be because of the fatty acid constituents of the fish oil (EPA and DHA) which resulted into the higher drug permeation for all the bigels (except SB3) as compared to hydrogels (SH1 and HH) as shown in Fig. 8. Bigel prepared from sodium alginate-fish oil displayed the highest cumulative drug permeation (0.9442 ± 0.0219 μg/cm^2) of all of the bigel formulations (Table 3). Among Bigels prepared from HPMC-fish oil HB1 showed the highest cumulative permeation (0.8072 ± 0.0108 μg/cm^2). The Higuchi model regression (r^2) values for all formulations are shown in Table 3.

4 Conclusion

Bigels prepared from polymer hydrogel and fish oil oleogel mixture were effective as vehicles for transdermal drug delivery as compared to hydrogel formulations (HH1 and SH1). Fish oil bigels offer better thermal stability and higher drug permeation than hydrogel formulations alone. Texture analysis and rheological characterization revealed ease in spreadability and pseudoplastic nature of the bigels. The addition of fish oil proved to beneficial as it allowed bigels to offer higher cumulative drug permeation and drug flux, which may be due to the omega-3 fatty acids (EPA and DHA). The oleogel-hydrogel proportion of 10:90 was proven to be the best combination for bigels in this study showing higher release and good mechanical properties. Thus, bigels can be introduced as a new drug delivery vehicle for pharmaceutical and cosmetic products but further studies are required to investigate more about the bigel technology and to channel it towards perfection.

Acknowledgement

The author would like to thank Centre For Research and

Instrumentation, and the Faculty of Pharmacy, Universiti Kebangsaan Malaysia in providing research grant UKM-GUP-2011-016 and miscellaneous support during this study.

Conflict of Interest

Authors have no conflict of interest to report.

References

1) Otto, W.; Drahoslav, L. Hydrophilic gels in biologic use. *Nature* **185**, 117-118(1960).
2) Allan, S. H. Hydrogels for biomedical applications. *Adv. Drug. Deliv. Rev.* **64**, 18-23(2012).
3) Vintiloiu, A.; Leroux, J. Organogels and their use in drug delivery- A review. *J. Control Release* **125**, 179-192(2008).
4) Abdallah, D. J.; Weiss, R. G. Organogels and low molecular mass organic gelators. *Adv. Mater.***12**, 1237-1247(2000).
5) Isabella, O.; Trere, R.; Luppi, B.; Bigucci, F.; Cerchiara, T.; Zuccari, G.; Zecchi, V. Hydrogels formed by cross-linked poly(vinyl alcohol)as sustained drug delivery system. *Arch. Pharm.* **335**, 89-93(2002).
6) Amin, M. A.; Abdel-Raheem, I. T. Accelerated wound healing and anti-inflammatory effects of physically cross linked polyvinyl alcohol-chiosan hydrogel containing honey bee venom in diabetic rats. Arch. Pharm. Res. DOI 10.1007/s12272-013-0308-y(2013).
7) Rehman, K.; Zulfakar, M. H. Recent advances in gel technologies for topical and transdermal drug delivery. *Drug. Dev. Ind. Pharm.* **40**, 433-440(2014).
8) Puglia, C.; Tropea, S.; Rizza, L.; Santagati, N. A.; Bonina, F. In vitro percutaneous absorption studies and in vivo evaluation of anti-inflammatory activity of essential fatty acis(EPA)from fish oil extracts. *Int. J. Pharm.* **299**, 41-48(2005).
9) Richard, H.; Thomas, C. P.; Bowen, J. L.; Heard, C. M. In vitro transcutaneous delivery of ketoprofen and polyunsaturated fatty acids from pluronic lecithin organogel vehicle containing fish oil. *J. Pharm. Pharmacol.* **58**, 903-908(2006).
10) Khayel, G.; Young, J.; Burns-Whitmore, B.; Spalidng, T. Effects of fish oil supplements on inflammatory acne. *Lipids Health Dis.* **11**, doi:10.1186/1476-1511X-1111-1165(2012).
11) Almeida, I. F.; Fernandes, A. R.; Fernandes, L.; Pena Ferreira, M. R.; Costa, P. C.; Bahia, M. F. Moisturizing Effect of Oleogel/Hydrogel Mixtures. *Pharm. Dev. Technol.* **13**, 487-494(2008).
12) Di Michele, L.; Fiocco, D.; Varrato, F.; Sastry, S.; Eiser, E.; Foffi, G. Aggregation dynamics, structure and mechanical properties of bigels. *Soft Matter.* **10**, 3633-3648(2014).
13) Rhee, G. J.; Woo, J. S.; Hwang, S. J.; Lee, Y. W.; Lee, C. H. Topical oleo-hydrogel preparation of ketoprofen with enhanced skin permeability. *Drug. Dev. Ind. Pharm.* **25**, 717-726(1999).
14) William, L.; David, K.; Brandon, S.; Peppas, A. Polymers for drug delivery systems. *Annu. Rev. Chem. Biomol. Eng.* **1**, 149-173(2010).
15) Luppi, B.; Bigucci, F.; Baldini, M.; Abruzzo, A.; Cerchiara, T.; Corace, G.; Zecchi, V. Hydroxypropyl methylcellulose films for prolonged delivery of the antispychotic drug chlopromazine. *J. Pharm. Pharmacol.* **62**, 305-309(2010).
16) Siepmann, J.; Peppas, N. A. Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose(HPMC). *Adv. Drug. Deliv. Rev.* **48**, 139-157(2001).
17) Amrajargal, S.; Tomas, S.; Vera, K.; Petr, S. On the characeterization of sodium alginate/gelatin based hydrogels for wound dressing. *J. Mech. Behav. Biomed. Mater.* **18**, 152-166(2013).
18) Hnin-Ei, T.; Zulfakar, M. H.; Shiow-Fern, N. Alginate based bilayer hydrocolloid films as potential slow-release modern wound dressing. *Int. J. Pharm.* **434**, 375-383(2012).
19) Thomas, C. P.; Heard, C. M. Probing the skin permeation of eicosapentanoic acid and ketroprofen: 2. Comparative depth profiling and metabolism of eicosapentanoic acid. *Eur. J. Pharm. Biopharm.* **67**, 156-165(2007).
20) Zulfakar, M. H.; Abdelouahab, N.; Heard, C. M. Enhanced topical delivery and ex vivo anti-inflammatory activity from a betamethasone dipropionate formulation containing fish oil. *Inflamm. Res.* **59**, 23-30(2010).
21) Huri, M. F. D.; Ng, S.-F.; Zulfakar, M. H. Fish oil-Based Oleogels: Physiochemicals Characterisation and In Vitro Release of Betamethasone Dipropionate. *Int. J. Pharm. Pharmaceut. Sci.* **5**, 458-467(2013).
22) Rehman, K.; Tan, C. M.; Zulfakar, M. H. Development and In-vitro Characterization of Fish Oil Oleogels Containing Benzoyl Peroxide and Salicylic Acid as Keratolytic Agents. *Drug Res.* **64**, 159-165(2014).
23). Fusako, T.; Kazuo, N.; Nagao, T.; Tomoko, Y.; Hideaki, K.; Takao, K.; Mitsumasa, M. Effects of docosahexaenoic acid in an experimental rat model of nonalcoholic steatohepatitis. *J. Oleo Sci.* **59**, 407-414(2010).
24) Teicher, B. A.; Ara, G.; Buxton, D.; Leonard, J.; Schaub, R. G. Optimal scheduling of interleukin-12 and chemotherapy in the murine MB-49 bladder carcinoma and B-16 melanoma. *Clin. Cancer Res.* **3**, 1661-1667(1997).

25) Graells, J.; Ojeda, R. M.; Garcia-Cruz, A. Effect of imiquimod as compared with surgery on the cancerization field in basal cell carcinoma. *Actas Dermosifiliogr.* **105**, 53-59(2014).
26) Tarbet, E. B.; Larson, D.; Anderson, B. J; Bailey, K. W.; Wong, M. H.; Smee, D. F. Evaluation of imiquimod for topical treatment of vaccinia virus cutaneous infections in immunosuppressed hairless mice. *Antiviral Res.* **90**, 126-133(2011).
27) Jin, J. Y.; Jung, Y. J.; Choi, S. S.; Hwang, J.; Chung, E. Autophagy mediated anti-tumoral of imiquimod in CaCo-2 cells. *Biochem. Biophys. Res. Com.* **386**, 455-458(2009).
28) Hadley, G.; Derry, S.; Moore, R. A. Imiquimod for actinic keratosis: systematic review and meta-analysis. *J. Invest. Dermatol.* **126**, 1251-1255(2006).
29) Ashok, P.; Domien, S.; Winnok, Vos.; Ans, L.; Koen, D. Preparation and rheological characterization of shellac oleogels and oleogel-based emulsions. *J. Colloid Interface Sci.* **411**, 114-121(2013).
30) Prasanthi, S.; Senthil, A.; Ahmad, M. Percutaneous absorption of triacyglycerols(TAGS), tocols and carotenoids: comparison studies of crude and refined palm oil. *MJPS.* **11**, 33-48(2013).
31) Julio, C. B. R.; Julice, D. L.; Maria, C. N. M.; Daniel, B. A.; Lizielle, M. R. G.; Rosiana, L. D. C. Thermal and rheological properites of orgnaogels formed by sugarcane or candelilla wax in soyabean oil. *Food Res. Int.* **50**, 318-323(2013).
32) United States Pharmacopoeia 37/National Formulary 32 USA, Rockville: United State Pharmaopoeial Convention Inc; 2014.