Colloids and Surfaces B: Biointerfaces 128 (2015) 155–164

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Modulating the properties of sunflower oil based novel emulgels using castor oil fatty acid ester: Prospects for topical antimicrobial drug delivery

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ARTICLE INFO

Article history:
Received 2 October 2014
Received in revised form 13 February 2015
Accepted 15 February 2015
Available online 21 February 2015

Keywords:
Bicontinuous
Pseudoplastic
Mechanical
Diffusion
Iontophoresis
Cell proliferation

ABSTRACT

The current study describes the effect of polyglycerol polyricinoleate (PGPR) on the properties of sunflower oil and span-40 based emulgels. The prepared emulgels contained PGPR in varied concentrations. The microstructure of the emulgels was characterized by bright-field microscopy. The molecular interactions amongst the components of the emulgels were studied using FTIR spectroscopy. The flow and mechanical behaviors of the emulgels were studied using cone-and-plate viscometer and static mechanical tester, respectively. The efficiency of the metronidazole-loaded emulgels as antimicrobial formulations was tested *in vitro*. *E. coli* was used as the model microorganism for the antimicrobial study. The emulgels were also explored for iontophoretic delivery applications. The biocompatibility of the emulgels was tested using human keratinocytes (HaCaT). The microscopic evaluation of the emulgels indicated formation of biphasic formulations. FTIR studies suggested a decrease in the hydrogen bonding amongst the components of the emulgels as the concentration of the PGPR was increased. Viscosity studies indicated shear-thinning property of the emulgels. An increase in the PGPR concentration resulted in the reduction in the mechanical properties of the emulgels. Incorporation of PGPR resulted in the decrease in the drug released (both passive and iontophoresis) from the emulgels. The emulgels were found to be cytocompatible in the presence of keratinocytes. The drug loaded emulgels showed good antimicrobial activity against *E. coli*. In gist, the developed emulgels can be tried for controlled delivery of antimicrobial drugs. The physical and the release properties of the emulgels can be modulated by incorporating PGPR in varied proportions.

1. Introduction

In recent years, there has been an increase in the issues related to the health concerns from the use of gels containing organic solvents. This has led to an increase in the research on gel-based system prepared using vegetable oils (*e.g.* palm oil, castor oil, sunflower oil, sesame oil). Sunflower oil (SO) is one of the commonly used vegetable oil and have been used in the production of formulations for pharmaceutical applications [1]. The use of sunflower oil is on the rise due to their easy availability and low price. Additionally, it does not elicit any cytotoxic effect on the skin cells. Recently, structuring of the vegetable oils has gained much importance. Structuring agents help in tailoring the properties of the emulgels. Nowadays, there is an increased trend in the use of low molecular weight organogelators (LMOG) over crystalline triacyglycerols (TAG) for structuring vegetable oils. The current study evaluates the effect of polyglycerol polyricinoleate (PGPR) on the properties of the emulgels prepared using span 40 and sunflower oil. Emulgels (emulsion gels) are a class of biphasic semi-solid formulation [2]. Currently, they are being tried for controlled delivery applications [3]. Due to the presence of both aqueous and non-aqueous phases, emulgels offer the capability of delivering both hydrophilic and lipophillic agents [4]. Though the emulgels are biphasic in nature, they are thermodynamically more stable as compared to emulsions. Due to the above-mentioned reasons, the emulgels provide the advantages of both emulsions (biphasic system) and gels (improved stability). In recent years, emulgels have gained importance in topical drug delivery. Emulgels helps

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http://dx.doi.org/10.1016/j.colsurfb.2015.02.026
0927-7765/

improving the penetration of the drugs into the systemic circulation through the skin and prevent first-pass metabolism. These properties of emulgels increase the bioavailability of the drugs. Also, an improved patient compliance has been reported due to the reduced dosing frequency [5]. Emulgels based formulations have been used for the delivery of antifungal drugs (*e.g.* clotrimazole) for the treatment of candidiasis and other fungal infections. Antimicrobial oils (*e.g.* thyme oil, balsam oil, myrrh oil, birch oil) has been used for the preparation of anti-bacterial emulgels [6].

PGPR belongs to a class of non-ionic synthetic surfactant. The HLB value of PGPR is 1.5 ± 0.5. Low HLB surfactant supports formation of water-in-oil type of emulsions. The molecular weight of PGPR is 1050 Da [7]. The esters of polyglycerol have been widely studied as emulsifiers in pharmaceutical industries. There are two groups of polyglycerol esters, often denoted by E-number, polyglycerol esters of edible fatty acids (E-475 or PGFA) and polyglycerol polyricinoleate (E-476, also known as PGPR). PGPR has been extensively studied due to its viscosity modifying property. It has also been used as an emulsifier, either alone or in combination with other emulsifiers. As per USFDA, the acceptable daily intake limit of PGPR is $\leq$4 g/kg [8]. Though PGPR is extensively used in pharmaceutical industries, there are no studies on the effect of the PGPR on the physical properties and the drug release behavior of the emulgels.

In the present study, we report for the first time, the modulation of the properties of sunflower oil and span 40 based emulgels using PGPR. The effect of PGPR concentration on the properties of the emulgels has been studied. The emulgels were explored as matrices for controlled delivery applications. Metronidazole (MZ), a topical antibiotic, was used as a model antimicrobial drug [9]. The ability of the emulgels to serve as delivery matrices for AC iontophoresis was also studied. Though DC iontophoresis devices are available, they suffer from electrode polarization. This results in the accumulation of the charges at the electrode–skin interface, which may not only lead to skin irritation but also, in some cases, skin burns. Hence, in this study, the formulations were explored as delivery matrices for AC iontophoresis. The molecular interactions amongst the emulgels components and physical properties were also studied in-depth. Since the emulgels are meant to be applied over skin, it is important to understand the cytocompatibility of the formulations in the presence of the skin cells (keratinocytes). Hence, cytocompatibility of the emulgels were tested in the presence of HaCat cells (keratinocyte cells).

2. Materials and methods

Sunflower oil (Gold winner®) was procured from NCS Industries Pvt. Ltd., Kakinada, India. Span 40 was purchased from Lobachemi, Mumbai, India. PGPR was received as a gift from Nestle, India. Metronidazole was a gift sample from Arti drugs Pvt., Ltd., India. Microbial culture of *E. coli* was obtained from NCIM, Pune, India. Dulbecco's phosphate buffer saline 1× (DPBS) and Trypsin EDTA solution 1× was procured from Hi-media laboratories, Mumbai, India. HaCaT cell line, procured from NCCS, Pune, India, was kindly provided by Prof. T.K. Maiti, Indian Institute of Technology, Kharagpur, India. Fetal bovine serum (FBS) was purchased from Gibco-BRL, USA.

2.1. Preparation method of PGPR emulgels

The composition of the prepared emulgels has been tabulated in Table 1. The emulgels were prepared by varying the concentrations of PGPR, Span 40, sunflower oil and water. Accurately weighed Span 40 and PGPR was first dissolved in sunflower oil (50 °C) to form a clear isotropic mixture. To the above mixture, water was added drop-wise, with continuous homogenization (1200 rpm) to form a milky-white emulsion. The hot emulsions were then cooled to room-temperature (25 °C) to form emulgels. The formation of the emulgels at room-temperature was confirmed by inverted-tube method. The drug (metronidazole) loaded emulgels were also prepared in a similar manner. The final concentration of metronidazole in the emulgels was 1% (w/w). The stability of the emulgels was tested by keeping the emulgels for 10 months at 30 ± 2 °C/65% ± 5% relative humidity (ICH guidelines). The evaluation was based on the visible signs of color change, degradation or phase separation. A digital ATC pH meter (EI instruments, model no: 132E, India) was used to determine the pH.

2.2. Characterization of the emulgels

2.2.1. Microstructural analysis

The microstructure of the emulgels were observed under bright-field microscope (LEICA-DM 750, Germany). Delsa Nano C, Beckman Coulter, UK was used to determine the droplet size of the emulgels. The operation of the instrument was based on the principle of dynamic light scattering technique at an angle of 165°. The experiment was conducted at 25 °C. All the analysis was carried out using semi-solid concentrated emulsion as per the reported literature [10].

2.2.2. FTIR studies

AlphaE ATR-FTIR, Bruker, USA was used for the FTIR analysis. 1 g of the emulgels were placed over the ZnSe crystal surface. The emulgels were scanned in the wavenumber range of 450–4000 cm^{-1}.

2.2.3. Viscosity and mechanical analysis

The viscosity profile of the emulgels were measured using cone-and-plate viscometer (Bohlin visco 88, Malvern, UK). The angle of the cone was 5.4° and the diameter of the plate was 30 mm. All the analysis was conducted at room-temperature. The type of flow of the emulgels was determined using Ostwald–de wale power law Eq. (1) [11]:

$$\eta = K \cdot \gamma^{n-1} \tag{1}$$

where, η = shear viscosity, K = consistency index, n = power law index and γ = shear rate.

TA-HD plus texture analyser (Stable Micro Systems Ltd., Surrey, UK) was used to study the mechanical properties of the emulgels. The study was conducted using a 30 mm flat probe. The probe was moved to a distance of 2 mm after a trigger force of 5 g. The stress relaxation profile was analyzed using Peleg's stress relaxation model (Eq. (2)). %SR of the formulations was calculated using Eq. (3).

$$\frac{(F_0 - F(t))t}{F_0} = k_1 + k_2 \cdot t \tag{2}$$

where, F_0 = maximum force, $F(t)$ = force at a particular time, k_1 = initial rate of relaxation, and k_2 = extent of relaxation.

$$\%SR = \left(\frac{F_0 - F_r}{F_0}\right) \times 100 \tag{3}$$

The viscoelastic property of the emulgels were analyzed using Weichert model (Eq. (4)). The residual force and the relaxation times were calculated using the model.

$$P(t) = P0 + P1 \cdot e^{-t/\tau_1} + P2 \cdot e^{-t/\tau_2} + P3 \cdot e^{-t/\tau_3} \tag{4}$$

where, $P(t)$ is the magnitude of the decaying force at time t; P_0 is the magnitude of the residual force; P_1, P_2 and P_3 are the relaxation modulus of the spring; τ_1, τ_2 and τ_3 are the relaxation time of the dashpot during the stress relaxation test.