Ultrasonication-Assisted Preparation and Characterization of Emulsions and Emulsion Gels for Topical Drug Delivery

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ABSTRACT: The current study describes the use of ultrasonication for the preparation of biphasic emulsions and emulsion gels for topical drug delivery. Sorbitan monostearate (SMS) was used as the surfactant for stabilizing the interface of sesame oil (apolar phase) and water (polar phase). Emulsions were formed at lower concentrations of SMS, whereas emulsion gels were formed at higher concentrations of SMS. The formulations were characterized by fluorescent microscopy, X-ray diffraction, viscosity, stress relaxation, spreadability, and differential scanning calorimetry studies. Fluorescence microscopy suggested formation of oil-in-water type of formulations. There was an increase in the viscosity, bulk resistance, and firmness of the formulations as the proportions of SMS was increased. The emulsion gels were viscoelastic in nature. Thermal studies suggested higher thermodynamic stability at higher proportions of either SMS or water. Metronidazole, a model antimicrobial drug, was incorporated within the formulations. The release of the drug from the formulations was found to be diffusion mediated. The drug-loaded formulations showed sufficient antimicrobial efficiency to be used as carriers for topical antimicrobial drug delivery. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

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INTRODUCTION

Emulsions are thermodynamically unstable biphasic formulations. This may be associated with the destabilization of the interfacial membranes, formed by emulsifiers, which ultimately leads to the rupture of the membrane layer, thereby resulting in the loss of the internal phase.[1] The stability of the emulsions may be improved by increasing the stability of this interfacial membrane layer. It has been noted that the method of emulsification plays an important role in improving the stability of the emulsions.[2] In general, the formation of metastable emulsions has been reported when external energy is supplied to the pre-emulsion mixtures. Commonly, energy may be provided either by mechanical agitation (stirrer, colloid mill, mixer, valve homogenizer) or by ultrasonic probes. In recent years, ultrasonication has evolved as one of the most easy-to-handle and powerful homogenization technique for formulating biphasic formulations.[3] Ultrasonic transducer-based homogenizers stimulate the piezoelectric crystals, attached to the ultrasonic probes, to oscillate at a very high speed (>20,000 cycles/s).[4] The pre-emulsion flows toward the ultrasonic probe during the contraction phase, whereas it is pushed away from the probe during the expansion phase. As the speed of the probe is much higher than the speed of the flow of the pre-emulsion, this results in the generation of microscopic shockwaves within the liquids. The cavities, so formed, collapse within a fraction of a second. This releases a large amount of energy within the pre-emulsion. This phenomenon helps in the formation of smaller droplets within a continuous phase.[5] The use of ultrasonication has been reported by many researchers for the preparation of emulsions (microemulsions/nanoemulsions).[6,7]

Emulsion gels may be defined as the biphasic formulations, similar to the emulsions, but the formulations are semisolid in consistency.[8] The stability of the emulsion gels is much better than the emulsions and may be explained by the semisolid nature of the formulations. This restricts the movement of the internal phase droplets, thereby reducing the chances of coalescence of the droplets.[9] In recent years, emulsion gels have gained much importance because of their ability to control the release profile of the incorporated drugs. The preparation and the properties of the emulsion gels using ultrasonic-assisted method have not been explored much yet.

In the current study, an attempt was made to prepare biphasic formulations with improved properties using ultrasonic homogenization. The formulations were tested for the *in vitro* delivery of the antimicrobial drugs. Sesame oil was used as the apolar phase for the preparation of the formulations. Sesame oil is obtained from the seeds of the plant *Sesamum indicum*. The oil has very good antioxidant properties because of the presence of sesamin, sesamolin, and sesamol.[10] Even though the oil is easily available in the Indian subcontinent, the use of the sesame oil in formulating pharmaceutical products is rare. Sorbitan monostearate (SMS) is a nonionic surfactant and is being commonly used in pharmaceutical and food industries.[11] Keeping a note of the above, it seemed justified to develop sesame oil and SMS-based formulations. Emulsions and emulsion gels were prepared and characterized thoroughly. Metronidazole was incorporated within the prepared formulations and their drug release properties were studied in-depth under *in vitro* conditions.

EXPERIMENTAL

Materials

Sorbitan monostearate (Loba Chemie, Mumbai, India), a nonionic surfactant, was used as an emulsifier. Edible grade sesame oil (Tilsona®; Recon Oil Industries Ltd., Mumbai, India) was

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used as apolar phase to prepare the biphasic formulations (emulsions and emulsion gels). Nutrient agar and dialysis tubing (MW cutoff: 60 kDa) were obtained from Himedia (Mumbai, India). Microbial culture of *Bacillus subtilis* (NCIM 2699) was obtained from National Collection of Industrial Microorganisms (NCIM) (Pune, India) for conducting the antimicrobial studies. Metronidazole was obtained from Aarti drugs (Mumbai, India) as a gift sample and was used as received. Milli-Q Ultrapure water was used throughout the study.

Methods

Preparation of Emulsions and Emulsion Gels

The formulations were developed by varying the proportions of SMS, sesame oil, and water. Initially, a pre-emulsion was prepared by homogenous mixing of the three components in a magnetic stirrer (500 rpm, 70°C). The pre-emulsion was subsequently homogenized using a probe sonicator (6 mm probe, Syclon ultrasonic cell crusher, SKL-250IIDN; Ningbo Haishu Sklon Electronics Instruments Company Ltd., Zhejiang, China), operating at a ultrasonic frequency of 20–25 KHz and 150 W. The sonication was carried out for 20 cycles (6 s on time and 2 s off). The homogenization was carried out in an ice-bath to compensate the heat generated.

Metronidazole (1%, w/w)-loaded emulsions and emulsion gels were prepared similarly. Accurately weighed metronidazole was uniformly homogenized (by ultrasonication) in sesame oil and used as the apolar phase. The rest of the procedure remained same. The prepared formulations were checked for their organoleptic behavior such as odor, color, texture, and pH. The nature of the formulations (oil-in-water or water-in-oil) was checked by dilution test.[12]

Stability Studies

The stability of the prepared emulsions and emulsion gels were checked by accelerated stability study and intermediate stability study. The accelerated stability testing was carried out by freeze–thaw thermocycling method reported elsewhere.[13] The intermediate stability of the formulations was studied by incubating the formulations at 30 ± 2°C/65 ± 5% RH for 6 months (ICH guidelines). The formulations were checked for any changes in the physical properties at regular intervals.[14]

Microscopic Evaluation

The microstructure of the emulsions and emulsion gels was studied using Fluorescence Stereo Microscope (M205 FA; Leica, Germany). Samples were prepared by incorporating an oil-soluble dye (0.1% fluoral yellow) in sesame oil. The thin smears were prepared on glass slides and were visualized under green filter. The fluorescence micrographs were further used to predict the droplet size distribution of the formulations.[13] The diameter of the droplets was measured by ImageJ 1.43U software (National Institute of Health, Maryland, USA).

X-ray Diffraction Study

The relative changes in the crystallinity of the emulsions and emulsion gels were predicted by X-ray diffraction studies (PW3040; Philips, Almelo, Holland). Monochromatized CuK_α radiation ($\lambda = 0.154$ nm) was used as the X-ray source and the spectrum was recorded in the range of 5°–50° 2θ at a scan rate of 2° 2θ/min.[15]

Mechanical Behavior

A cone-and-plate viscometer (Bohlin visco 88; Malvern, Worcestershire, UK) was used for the viscosity measurements. During the measurements, the viscosity of the emulsions and emulsion gels were recorded at room temperature (RT, 25.0°C) in the shear rate range of 10–100 s^{-1}.[16]

Large-scale deformation properties (stress relaxation and spreadability) of the formulations were studied using Texture Analyser (TA-HDplus; Stable Microsystems, Godalming, UK).[17] The test parameters are enlisted in Table S1.

Thermal Analysis

Drop-ball method is the most commonly used method to determine the melting point (T_m) of the semisolid formulations. The melting point of the emulsion gels was determined using melting point apparatus-931 (EI Instruments, Haryana, India).[18]

The thermal profile of the formulations was studied using differential scanning calorimetry (DSC 200F3 Maia; Netzsch, Selb, Germany). Samples were accurately weighed (~15 mg) in the aluminum pans and were sealed with pierced lids. The test was carried out under inert atmosphere by purging nitrogen gas at a flow rate of 40 mL/min. The heating and the cooling thermal profiles were recorded in the temperature range from 25°C to 150°C at a scan rate of 5°C/min.[15]

Biocompatibility Study

Tetrazolium-based MTT [3-(4,5-dimethyltiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay was performed to estimate the cytocompatibility of the developed formulations using HaCaT cells.[19] The leachants of the formulations were collected in phosphate buffer saline (pH 7.2). 1.0×10^4 cells per well of the cell suspension were seeded in 96-well plates and incubated for 24 h to allow cell adhesion. Thereafter, 20 μL of the leachant of the formulations was added and further incubated for 24 h. The cell viability was then measured using MTT assay as per the reported literature.[20,21]

In Vitro Drug Release Studies

The release of metronidazole from the metronidazole-loaded formulations was studied *in vitro* using two-compartment modified Franz's diffusion cell. The receiver compartment contained 50 mL of phosphate buffer saline (pH 7.2). The release media was completely replaced with an equivalent amount of the fresh media at regular intervals of time. The replaced release media was analyzed at 321 nm using UV–Vis spectrophotometer (UV 3200 Double Beam; Labindia, Thane (West), Maharashtra, India). The study was conducted for 12 h.[22,23]

Antimicrobial Assay

The prepared emulsions and emulsion gels were evaluated for their antimicrobial efficiency against a gram-positive *Bacillus subtilis* according to the method reported elsewhere. The zone of inhibition for the metronidazole-loaded formulations was compared against commercially available metronidazole gel (Metrogyl®).[15]

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