Vol 8 | Issue 2 | 2018 | 21-34.

International Journal of Advanced Pharmaceutics

e-ISSN 2249 – 7706
print-ISSN 2249– 7714

www.ijapjournal.com Research Article

OFLOXACIN MICROEMULSION

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Access this article online

Home page: http://ijapjournal.com/

DOI: http://dx.doi.org/10.21276/ijap.2018.8.2.2

Quick Response code

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Received:15.6.18 **Revised**:25.06.18 **Accepted**:07.07.18

ABSTRACT

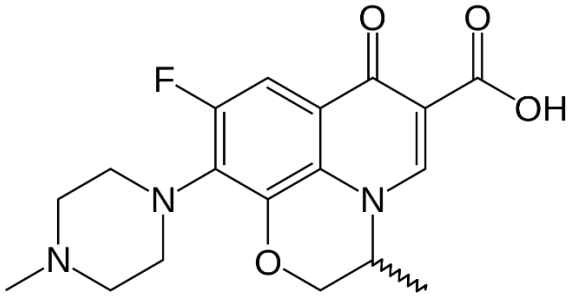
Microemulsions are thermodynamically stable, but are only found under carefully defined conditions. One way to characterize these systems is by whether the domains are in droplets or continuous. Characterizing the systems in this way results in three types of microemulsions: oil-in-water (o/w), water-in-oil (w/o), and bicontinuous. Generally, one would assume that whichever phase was a larger volume would be the continuous phase, but this is not always the case. Surfactants are molecules that typically contain a polar head group and an polar tail. They are surface-active and microstructure forming molecules with a strong chemical dipole. They can be ionic (cationic or anionic), nonionic, or zwitter ionic. Surfactant molecules self-associate due to various inter and intra molecular forces as well as entropy considerations. All of these serve to optimize the free-energy overall. For example, when surfactant is mixed with oil and water, they accumulate at the oil/water interface, because it is thermodynamically favorable. The surfactant molecules can arrange themselves in a variety of shapes. They can form spherical micelles, rod-shaped micelles, a hexagonal phase (consisting of rod-shaped micelles), lamellar (sheet) phases, reverse micelles, or hexagonal reverse micelles.

Keywords: Ofloxacin, Microemulsion, Formulation.

INTRODUCTION

Ofloxacin

Fig: 1. Structure of Ofloxacin



IUPAC Name: (*RS*)-7-fluoro-2-methyl-6-(4-methylpiperazin-1-yl)-10-oxo-4-oxa-1-azatricyclo[7.3.1.$0^{5,13}$]trideca-5(13),6,8,11-tetraene-11-carboxylic acid

Mol weight: 361.36 g/mol

Formula: $C_{18}H_{20}FN_3O_4$

Mechanismof Action: Ofloxacin acts on DNA gyrase and toposiomerase IV, enzymes which, like human topoisomerase, prevents the excessive supercoiling of DNA during replication or transcription. By inhibiting their function, the drug thereby inhibits normal cell division.

Bio Availability: 85-95%

Protein Binding: 32%

Half life: 8-9hr

Categories: Anti Bacterial, Anti Infective agents, Quinolines, Anti Infective agents,

Dose: 3mg/ml, 300 and 400 mg tablet

MATERIALS AND METHODS

Ethyl Oleate

CA Index Name: 9-Octadecenoic acid (9Z)-, ethyl ester
Other Names: 9-Octadecenoic acid (Z)-, ethyl ester; Oleic acid, ethyl ester (6CI,8CI); Crodamol EO; Esterol 123; Ethyl (Z)-9-octadecenoate; Ethyl cis-9-octadecenoate; Ethyl oleate; Nikkol EOO; Nofable EO 99
Molecular Formula: $C_{20}H_{38}O_2$
Molecular Weight: 310.51 g/mol
Physical State: Colorless to light yellow oily liquid
Boiling Point: 190°C
Melting Point: -32°C
Specific Gravity: 0.87

Ethyl oleate is primarily used as a vehicle for intramuscular injections. It is used as a solvent for drugs that are formulated in biodegradable capsules for subdermal implantation [1]. Ethyl oleate is known for being used in microemulsions for the delivery of cyclosporine [2]. It is a suitable solvent for steroids and lipophilic drugs, and has properties similar to almond and peanut oils, but is less viscous than fixed oils and more rapidly absorbed by body tissues [3].

Crodamol PMP

CA Index Name: Poly[oxy(methyl-1,2-ethanediyl)], -(1-oxopropyl)- -(tetradecyloxy)-
Other Names: Crodamol PMP; Polypropylene glycol 2-myristyl ether propionate
Molecular Formula: $(C_3H_6O)nC_{17}H_{34}O_2$
Physical State: Colorless oily liquid Boiling Point: N/A
Melting Point: N/A
Specific Gravity: 0.88
Solubility: Insoluble in water

Poly[oxy(methyl-1,2-ethanediyl)], **α**-(1-oxopropyl)-**ω**-(tetradecyloxy)- (PMP) has excellent emollient properties, which makes it a suitable replacement for mineral oil in many formulations. It is a thin liquid, which has good spreading properties and is an excellent solvent.

Crodamol-GTCC

CA Index Name: Glycerides, C8-10
Other Names: Akoline MCM; Akomed E; Arlamol M 812; C8-10 glycerides; Campul MCM; Capric/caprylic triglyceride; Capric/caprylic triglycerides; Caprylic/capric triglyceride; Caprylic/capric triglycerides; Captex 300; Captex 300 Low C6; Captex 300EP; Captex 335; Captex 355; Coconad MT; Coconard MT; Crodamol GTCC; Crodamol PC-DAB 10(S); Delios 325; Delios SK; Delios V; Delios V MCT oil; Delios VK koscher; Dermol M 5; Estasan 3575; Estasan GT 8-60; Estasan GT 8-65; Estol 1527; Estol 3601; Estol 3603; Ethox 2156; Labrafac CCTG; Labrafac LIPO WL 1349; Labrafac Lipophile; Labrafac Lipophile WL 1349; Labrafac WL 1349; Lexol GT 865; Liponate GC; Lumulse CC 33K; Miglyol 810; Miglyol 810N; Miglyol 812; Miglyol 812N; Miglyol 812S; Myritol 312; Myritol 314; Myritol 318; Myritol 325; Neobee M 5; Neobee O; Neobee Oil M 5; Neoderm TCC; Nikkol Triester F 810; O.D.O.; Panacet 810; Panacete; Panacete 810; Radia 7104; Rofetan GTCC; Rylo TG 50; Sefol 880; Stepan 108; Stepan-Mild GCC; Sun Crystal; Surfac MCTG; Tegosoft CT; Triester F 810; Triglycerides, C8-10; Velsan CCT
Physical State: Colorless, thin, slightly yellow, oily liquid
Boiling Point: N/A
Melting Point: 0°C Specific Gravity: 0.945
Solubility: Practically insoluble in water, miscible with long-chain hydrocarbons and triglycerides, soluble in acetone, ethanol and isopropanol

GTCC is a vehicle that is stable to oxidation. GTCC has good solvent properties. It contains medium chain triglycerides that can be used as a lipid nutritional source in oral and parenteral supplements. These medium-chain triglycerides consist mainly of completely saturated fatty acids, which give rise to its very low viscosity. GTCC has very low reactivity with sensitive pharmaceutical additives. These medium-chain lipids are absorbed into the portal system upon ingestion and are metabolized in the liver. They are transported as free fatty acids bound to albumin [4]. GTCC is used in many ways in pharmaceutical and healthcare settings which include the following: a solvent for active compounds in topicals, a water resistant topical vehicle, a dispersing aid, a melting point modifier in rectal suppositories, a mineral oil replacement, a crystallization inhibitor for oral solutions, a fill material for capsules, a plasticizer, a dispersion vehicle for colorants, a polishing aid, a tablet lubricant, a lipid source in nutritional products, and a parenteral vehicle [5].

Labrafac Lipophile WL 1349

CA Index Name: Glycerides, C8-10
Other Names: Akoline MCM; Akomed E; Arlamol M 812; C8-10 glycerides; Campul MCM; Capric/caprylic triglyceride; Capric/caprylic triglycerides; Caprylic/capric triglyceride; Caprylic/capric triglycerides; Captex 300; Captex 300 Low C6; Captex 300EP; Captex 335; Captex 355; Coconad MT; Coconard MT; Crodamol GTCC; Crodamol PC-DAB 10(S); Delios 325; Delios SK; Delios V; Delios V MCT oil; Delios VK koscher; Dermol M 5; Estasan 3575; Estasan GT 8-60; Estasan GT 8-65; Estol 1527; Estol 3601; Estol 3603; Ethox 2156; Labrafac CCTG; Labrafac LIPO WL 1349; Labrafac Lipophile; Labrafac Lipophile WL 1349; Labrafac WL 1349; Lexol GT 865; Liponate GC; Lumulse CC 33K; Miglyol 810; Miglyol 810N; Miglyol 812; Miglyol 812N; Miglyol 812S; Myritol 312; Myritol 314; Myritol 318; Myritol 325; Neobee M 5; Neobee O; Neobee Oil M 5; Neoderm TCC; Nikkol Triester F 810; O.D.O.; Panacet 810; Panacete; Panacete 810; Radia 7104; Rofetan GTCC; Rylo TG 50; Sefol 880; Stepan 108; Stepan-Mild GCC; Sun Crystal; Surfac MCTG; Tegosoft CT; Triester F 810; Triglycerides, C8-10; Velsan CCT
Physical State: Colorless viscous liquid
Boiling Point: > 150°C

Melting Point: N/A
Specific Gravity: 0.93 – 0.96 Insoluble in water
Soluble in ethanol, chloroform, methylene chloride, and vegetable oils

Labrafac is a non-rancidable fluid that has solubilizing properties. It is a vehicle used in both oral and topical formulations. Generally, it is used as an excipient for soft gelatin capsules, as an anti-sticking agent for tablets, and as an oily phase for W/O or O/W emulsions. It is made up of capric and caprylic triglycerides. Medium-chain triglycerides have a wide variety of uses orally, parenterally, and topically. They can be used as a base for oral emulsions of drugs that are unstable in aqueous media. They have also been shown to enhance intestinal absorption [6]. Medium-chain triglycerides can act as fillers and lubricants in capsules and sugar-coated tablets. In combination with long-chain triglycerides, they can be used for total parenteral nutrition IVs. These nutritional agents can be used for diseases involving the malabsorption of fats. Medium-chain triglycerides offer many advantages over long-chain triglycerides. This includes better spreading properties, no impedance of skin respiration, good penetration and emollient properties, no visible film on skin surface, good compatibility with other components and drugs, good solvent properties, and good stability against oxidation [6].

Oleic Acid
CA Index Name: 9-Octadecenoic acid (9Z)-
Other Names: 9-Octadecenoic acid (Z)-; Oleic acid (8CI); 9-Octadecenoic acid, (Z)-; 9-cis-Octadecenoic acid; 9Z-Octadecenoic acid; B 115; Clear FRAC EF; Crodacid O-P; Crossential O 94; D 100; D 100 (fatty acid); Edenor ATiO5; Edenor FTiO5; Emersol 205; Emersol 211; Emersol 213NF; Emersol 214NF; Emersol 233; Emersol 6313NF; Extra Oleic 80R; Extra Oleic 90; Extra Oleic 99; Extra Olein 80; Extra Olein 90; Extra Olein 90R; Extra Olein A 1981; Industrene 105; Lunac O-CA; Lunac O-LL; Lunac O-P; Lunac O-V; Lunac OA; NAA 35; NAA 38; Neo-Fat 92-04; Oleine 7503; Pamolyn 100; Priolene 6204; Priolene 6906; Priolene 6907; Priolene 6928; Priolene 6930; Priolene 6933; Vopcolene 27; Wecoline OO; Z-9-Octadecenoic acid; cis-9-Octadecenoic acid; cis-Oleic acid; cis- 9-Octadecenoic acid; 9-cis-Octadecenoic acid; 9-cis-
Molecular Formula: $C_{18}H_{34}O_2$
Molecular Weight: 282.46 g/mol Physical State: Pale yellow oily liquid Boiling Point: 360°C
Melting Point: 16.3°C
Specific Gravity: 0.895 Insoluble in water
Oleic acid is used as an emulsifying agent in food products and topical pharmaceutical formulations, mostly as a penetration enhancer. It can be used to improve the bioavailability of poorly water-soluble drugs in tablets, or as part of the vehicle for soft gelatin capsules. Oleic acid can be radiolabeled with ^{131}I and ^{3}H and used in medical imaging as well [7].

Dioctyl Sodium Sulfosuccinate (DOSS)
CA Index Name: Butanedioic acid, 2-sulfo-, 1,4-bis(2-ethylhexyl) ester, sodium salt (1:1)
Other Names: Aerosol OT-B (6CI); Butanedioic acid, sulfo-, 1,4-bis(2-ethylhexyl) ester, sodium salt (9CI); Succinic acid, sulfo-, 1,4-bis(2-ethylhexyl) ester, sodium salt (8CI); 05035TX; 1,4-Bis(2-ethylhexyl) sodium sulfosuccinate; AOT; AOT 100; AOT I; Acrosol OT 100; Adekacol EC 4500; Adekacol EC 8600; Aerosol A 501; Aerosol AOT; Aerosol GPG; Aerosol OT; Aerosol OT 100; Aerosol OT 70; Aerosol OT 70PG; Aerosol OT 75; Aerosol OT 75E; Aerosol OT 75PG; Aerosol OT 94; Aerosol OT-A; Aerosol OT-S; Airrol CT 1; Airrol CT 1L; Airrol OP; Alcopol O; Alkasurf SS-O 75; Alphasol OT; Astrowet 608; Astrowet O 70PG; Astrowet O 75; B 80; Berol 478; Bis(2-ethylhexyl) S-sodium sulfosuccinate; Bis(2-ethylhexyl) sodiosulfosuccinate; Bis(2-ethylhexyl) sodium sulfosuccinate; Bis(2-ethylhexyl) sulfosuccinate sodium salt; Carabon DA 72; Celanol DOS 65; Celanol DOS 75; Chemax DOSS 70PG; Colace; Comfolax; Complemix; Constonate; Coprol; Coprola; Correctol Stool Softener Laxative; DESS; DOSS; DOSS 70; DSS; Defilin; Di(2-ethylhexyl) sulfosuccinate sodium salt; Di-2-ethylhexyl sodium sulfosuccinate; Dialose; Dioctlyn; Dioctyl; Dioctyl sodium sulfosuccinate; Dioctyl sulfosuccinate sodium; Dioctyl sulfosuccinate sodium salt; Dioctyl-Medo Forte; Dioctylal; Diomedicone; Diosuccin; Diotilan; Diovac; Diox; Disonate; Disponil SUS-IC 875; Disposaject; Docusate sodium; Doxinate; Doxol; Drewfax 007; Dulcivac; Dulsivac; Duosol; E 480; Emcol 4500; Empimin OP 70; Empimin OT 75; Eurowet PG; Freetex OT; Gemtex SC; Gemtex SC 40; Genopur SB 1970J; Geropon DOS; Geropon SDS; Geropon SS-O 75; Geropon WT 27; Humifen WT 27G; Isoprotanc; KM 10-1610; Karawet DOSS; Konlax; Koremul 290; Kosate; Lankropol 4500; Lankropol KO 2; Laxinate; Leveling Agent T; Leveller T; Lipal 860K; Lipal 870P; Lumiten I-RA; Lutensit A-BO; Lutensit A-BOS; M 75; Mackanate DOS 75; Manoxol; Manoxol OP; Manoxol OT; Marlinat DF 8; Mervamine; Miconate DOS; Modane Soft; Molatoc; Molcer; Molofac; Monawet M 085P; Monawet MO 65-150; Monawet MO 70; Monawet MO 70E; Monawet MO 70R; Monowet MO 70R; Monowet MO-E 75; NK-EP 70G; Neocol P; Neocol SW-C; Neocol YSK; Neopelex OTP; Nevax; Newcol 290M; Newcol 291EG; Newcol 291M; Newcol 291PG; Newkalgen EP 60P; Newkalgen EP 70G; Nikkol OTP 100; Nikkol OTP 100S; Nikkol OTP 70; Nikkol OTP 75; Nissan Rapisol; Nissan Rapisol A 30; Nissan Rapisol A 80; Nissan Rapisol A 90; Nissan Rapisol B 07; Nissan Rapisol B 30; Nissan Rapisol B 80; Nissan Rapisol B 90; Nonit; Norval; OT; OT 100; OT 70; OT75; OT 75 (surfactant); OT 75E; OTP 100; OTP 75; Obston; Octowet; PAV 1019; Pelex OT; Pelex OT-P; Pentex 99; Persol KMN 3; Pionin A 51B; Rapisol; Rapisol A 30; Rapisol A 80; Rapisol A 90; Rapisol B 30; Rapisol B 80; Rapisol B 90; Regutol; Revac; Rewopol SBDO 70; Rikakoru M 75; Rikasafu G 30; SBO; SN-Wet OT 70; SV

102; Sanmorin OT; Sanmorin OT 70; Sanmorin OT 70N; Sanseparer 100; Secosol DOS 70; Sobital; Sodium 1,2-bis(2-ethylhexyloxycarbonyl)-1-ethanesulfonate; Sodium 1,4-bis(2-ethylhexyl) sulfosuccinate; Sodium bis(2-ethylhexyl) sulfosuccinate; Sodium bis(ethylhexyl) sulfosuccinate; Sodium di(2-ethylhexyl) sulfosuccinate; Sodium di(ethylhexyl) sulfosuccinate; Sodium dioctyl sulfosuccinate; Sodium docusate; Softil; Soliwax; Solovet; Solusol; Sorpol 5050; Spilon 8; Succinate STD; Sulfimel DOS; Sulfosuccinic acid bis(2-ethylhexyl)ester sodium salt; Sulfosuccinic acid di-2-ethylhexyl ester sodium salt; Sunnol LDF 110; Surfonic DOS 75PG; TKB 20; Talosurf; Tex-Wet 1001; Triton GR 5; Triton GR 5M; Triton GR 7; Triton GR 7M; Triton GR 7ME; Vatsol OT; Velmol; Warcowet 060; Waxsol; Wetaid SR; Yal
Molecular Formula: $C_{20}H_{37}SO_7Na$
Molecular Weight: 444.57 g/mol
Physical State: White waxy solid
Melting Point: Decomposes Specific Gravity: 1.1
Easily soluble in methanol, diethyl ether, and glycerin Partially soluble in hot water, Very slightly soluble in cold water.

Butanedioic acid, 2-sulfo-, 1,4-bis(2-ethylhexyl) ester, sodium salt (DOSS) is an ionic surfactant. It is generally used as an emulsifying, wetting, dispersing, or solubilizing agent for external use. Therapeutically, DOSS is used as a stool softener. Its surface-active characteristics allow for the management of constipation [8, 9]. DOSS has also been reported to increase intestinal absorption of drugs [10].

Formulation of Microemulsions

Microemulsions are formulated by mixing various components such as oil, surfactant, co-surfactant (if needed), and water in various proportions. The concentration of various mixtures may be plotted in a ternary-phase diagram or a pseudo-ternary phase diagram to identify compositions that form visually clear microemulsions. Formulations were prepared using ethyl oleate (EO), Crodamol GTCC® (GTCC), Labrafac Lipophile WL 1349®, oleic acid, and Crodamol PMP® (PMP). Appropriate quantities of docusate sodium (DOSS) and oil (w/w) were weighed out on a PB303-S/FACT balance (Mettler Toledo) and were mixed overnight to form mixtures of weight rations 1:5, 1:10 and 1:15. Once completely dissolved, aliquots were taken from the surfactant/oil stock solution and titrated with water [15-18]. An aliquot of the surfactant/oil mixture was weighed in screw-cap test tube. Reverse osmosis (RO) water was added drop-wise and the weight was recorded after the addition of each drop. After recording the weight, samples were vortexed for 15 seconds at 1600 rpm and examined for visual clarity. Visual observations were performed for each addition of water. When turbidity that did not disappear after vortexing was observed the sample was stored at room temperature for 24 hours. If the turbidity disappeared after the 24 hour equilibration time more water was added drop wise and the procedure repeated till turbidity appeared. Addition of water was stopped when the turbidity was stable beyond 24 hours. The total amount of water incorporated into a stable microemulsion was determined by summing the water increments until the appearance of turbidity. These weights of water, surfactant and oil were transformed into relative percentages, and these percentages were plotted on a ternary phase diagram using Sigmaplot® software. Surfactant/oil mixtures were prepared in concentrations of 1:4 and 1:7 for EO and PMP. These were prepared in the same manner as previously described, titrated similarly with water, and the data obtained were used in the ternary phase diagrams.

Buffer and pH Effects

Buffer and pH effects on the amount of water incorporated into the microemulsion were examined using a method similar to formulation of microemulsions with RO water. Acetate buffers (pH = 4, 5 and 6) and phosphate buffers (pH = 7, 8, 9, 10 and 11) as well as DOSS/EO and DOSS/PMP in a surfactant/oil ratio of 1:10 were used in the experiment. Aliquots of the surfactant/oil mixture were weighed on an analytical balance and titrated with the various buffer solutions. Each surfactant/oil mixture was titrated with each of the 8 buffers. All experiments were done in triplicate. Maximum percent buffer incorporated was plotted vs. pH for each of the two, surfactant/oil combinations.

Polarized Light Microscopy

Samples were examined under polarized and non-polarized light. Surfactant/oil mixtures of DOSS/EO and DOSS/PMP in concentrations of 1:15 were used. An aliquot of the surfactant/oil mixture was taken and a sufficient amount of water was added to achieve the desired water/surfactant molar ratio (Wo = [H2O]/[DOSS]) [19, 20]. The desired Wo's were 25, 75, and 95 for PMP, and 25, 30 and 195 for EO. A drop of the sample was placed on a slide and observed using a 40x objective on a Nikon model TiU coupled with photometric Coolsnap EZ 20 MHz monochrome camera, and was controlled by MetaMorph software. Each sample was observed and pictures taken under normal optics with and without a polarizing filter to determine if the samples exhibited optical birefringence.

Conductivity

Conductivity studies were performed using a Mettler Toledo Seven Multi with InLab 741 conductivity probe. The probe had a cell constant of 0.102919 cm^{-1} with a range from 0.001 μS/cm to 500 μS/cm. Samples were prepared (in triplicate) of DOSS/EO and DOSS/PMP ratios of 1:5, 1:10, and 1:15. Water was added, using an analytical balance, to achieve various desired Wo's ranging from 2.5 to 95. The conductivity probe was placed in approximately 10 mL of sample and conductivity measured. After measurements, the probe was rinsed with

RO water, soaked in approximately 200 mL fresh RO water for five minutes, rinsed again with RO water, dried, and then used for next sample. This cleaning procedure was performed after each sample reading.

Rheology

Rheology experiments were performed on an AR-2000ex (TA Instruments). Microemulsion samples with Wo = 2.5, 5, and 7.5 were prepared using 1:10 DOSS/EO and RO water. Geometries of the instrument were 60-mm 1-degree Al cone with Peltier plate**,** and recessed rotor with concentric cylinder jacket. Temperature was controlled at 20°C with the Peltier plate and concentric cylinder jacket. Dynamic and steady flow testing were performed on the samples. Dynamic frequency sweep was performed at 20°C from 10 to 0.1 rad/sec with an oscillatory stress of 0.01 Pa. Dynamic time sweep was performed at 20°C, 0.4 rad/sec, and an oscillatory stress of 0.01 Pa. Steady state flow was performed at 20°C from 0.1 to 1E5 μN-m, 1000 sec^{-1} maximum, then 1000 to 0.1 sec^{-1}.

Dynamic Light Scattering

Dynamic light scattering (DLS) experiments were performed to determine particle size of the microemulsions. DOSS/EO and DOSS/PMP microemulsions of 1:5, 1:10, and 1:15 concentrations with various Wo values were prepared. Microemulsion samples were transferred into 6x50 mm Durex Borosilicate Glass Culture Tubes (VWR Scientific Products) and centrifuged for five minutes at 23°C and 5000 rpm using an Eppendorf 5430R centrifuge. Culture tubes were then placed in the Nicomp 380 ZLS (Particle Sizing Systems, Santa Barbara, CA) and particle size determined. Three cycles were run at 30 minutes per cycle for each sample, with a channel width of 5.5 μSec, temperature of 23°C, and 90° scattering angle. Mean volume weighted diameter was then calculated from the three runs for each sample.

Development of Sterilization Method

An aseptic filtration method was developed for the DOSS/EO microemulsion systems that show potential to be applied to other microemulsion systems. Microemulsions show great potential to be used as an ophthalmic dosage form. Microemulsions can increase the solubility of a wide variety of drugs and enhance that drug's absorption in the eye [11]. Many inherent properties of a microemulsion (low viscosity, transparency, thermodynamic stability, etc.) prove to be very advantageous for ophthalmic administration [12]. Currently, CMV retinitis in AIDS patients is treated with chronic antiviral therapies (due to the drugs' virustatic abilities) including intravenous injections, intravitreal injections, or intraocular implants. This proves to be a difficult and not an ideal method to deliver the drug. An ocular microemulsion dosage form of the drug could prove useful in a situation such as this. Sterility of a product is required for ocular use, so an aseptic filtration method was developed for the DOSS/EO microemulsion system. Due to the relatively small amounts of water in the microemulsions, the samples couldn't be sterilized by autoclaving. A filtration method was therefore developed for the formulations due to the low viscosity and ease of filtration without the use of elaborate equipment.

All glassware used were packaged appropriately, autoclave tape applied on the wrap, and were autoclaved at 15 psi and 121°C for 15 minutes. Mueller Hinton (MH) Agar plates were then prepared by dissolving the dehydrated agar in RO water followed by autoclaving. After autoclaving and cooling slightly, aseptic technique was used to transfer approximately 20 mL MH Agar solution into presterilized monoplates. The MH Agar cooled in the plates, which were then refrigerated until ready to use. When ready for use, the MH Agar plates were equilibrated to room temperature (RT) by maintaining at RT for approximately 30 minutes.

Microemulsions were prepared using 1:5 DOSS/EO and a sufficient amount of sterile water for injection to achieve Wo's of 2.5, 10, and 15. The surfactant/oil mixture was measured out and sufficient water was added using an analytical balance. The solution was vortexed for 15 seconds at 1600 rpm and allowed to equilibrate for 24 hours at room temperature. After system equilibration, 2 mL of the sample was withdrawn into a sterile syringe in a laminar hood and approximately 100 μL passed through a 0.2 μm Nalgene® syringe filter (25 mm surfactant free cellulose acetate membrane) directly onto the plate. After allowing absorption into the agar for approximately one hour in the laminar hood, plates were then closed and placed in an incubator at 35°C. The plates were then checked daily for any growth. This was repeated in duplicate.

Small squares of the cloudy portions of MH agar were cut out and placed in test tubes containing MH broth. Samples of the microemulsions were also directly introduced into test tubes containing MH broth. All tubes were vortexed and incubated at 35°C. Samples were taken from the tubes after approximately 24 hours, were gram stained, and examined under a microscope for bacteria.

EO alone as well as the 1:5 DOSS/EO mixture were plated on MH Agar plates. 50 μL of unfiltered EO, unfiltered DOSS/EO, filtered EO, and filtered DOSS/EO were plated in a laminar hood and incubated at 35°C. The EO and DOSS/EO samples were filtered using the same syringe filter as the microemulsion samples. The plates were checked daily for signs of growth. This experiment was done in duplicate.

Validation of Sterilization Method

Validation of the sterilization method was done using direct inoculation and membrane filtration. Fluid thioglycollate medium (FTM) and tryptic soy broth (TSB) were prepared, and used for direct inoculation and membrane filtration. All tests were carried out under aseptic conditions in a laminar airflow hood.

For direct inoculation, there was a negative control, two positive controls, and three samples. The negative control contained 10 mL of the uninoculated medium. The positive controls were prepared using E. coli. E. coli ATCC 25922 was cultured on an MH Agar plate and incubated at 35°C for approximately 24 hours. Bacterial colonies on the plate were swabbed and placed into a test tube containing 5 mL of sterile saline. The bacterial concentration was determined using a Spectronic 20 Genesys Spectrophotometer (Spectronic Instruments) at 625 nm. Sterile saline was used as a blank, and the sample absorbance was compared to a 0.5 McFarland Standard (1.5 CFU/mL). Serial dilutions were performed to achieve dilutions of 10^{-4} and 10^{-8} CFU/mL. The first positive control contained 9.9 mL of the medium and 100 µL of the 10^{-4} dilution. The second positive control contained 9.9 mL of the medium and 100 µL of the 10^{-8} dilution. The three samples test tubes contained 9 mL of the medium and 1 mL of the sample that had been passed through a 0.2 µm Nalgene® syringe filter (25 mm surfactant free cellulose acetate membrane) attached to a syringe. The microemulsion samples were identical to the formulations used in method development involving sterilization by aseptic filtration (1:5 DOSS/EO microemulsions with Wo = 2.5, 10, and 15). FTM samples were incubated at 35°C. One set of TSB samples was incubated at 35°C, and another set of TSB samples was incubated at room temperature (approximately 20°C). This totaled 18 test tubes for direct inoculation.

For membrane filtration, there was a negative control, two positive controls, and three samples. The negative control contained 10mL of uninoculated medium. A Millipore® 25 mm glass microanalysis vacuum filter holder with fritted glass support filtration apparatus was assembled. The receptacle for the fluid to be tested was held to the fritted glass support with an anodized aluminum spring clamp. A No. 5 stopper attached the apparatus to a 125 mL filter flask with a vacuum pump connection. Millipore nitrocellulose membrane filters of 0.22 µm pore size were placed on the support base and 500µL of the sample was placed in the receptacle. Formulations identical to those used in direct inoculation were used for membrane filtration. The sample was filtered by pipetting the sample onto the filtration unit placed, and vacuum was applied to facilitate filtration. After the sample passed through the membrane completely, the membrane was washed twice with sterile water. Filters were removed from the apparatus, cut into 3 pieces of approximately equal surface area and placed into test tubes containing 10mL of medium (FTM, TSB at 35°C, and TSB at room temperature). There were two positive controls. For the first positive control, 200 µL of the 10^{-4} E. coli dilution was added to 1.8 mL of sterile saline in a test tube. The membrane filter was first wet with sterile saline, and then 1mL of the first positive control solution was placed into the receptacle to be filtered. After the solution filtered through, the membrane was washed with sterile water twice. The filter was then removed and treated similarly as with the microemulsion samples described earlier. The same procedure was followed for 200 µL of the second positive control, as with the first positive control. This totaled 18 test tubes for membrane filtration.

Microemulsion samples of 400 µL were withdrawn from each of the 36 tubes for direct inoculation and filtration on Days 0, 7 and 14. These samples were transferred into Eppendorf microcentrifuge tubes and then spiroplated onto blood agar plates using a SpiralBiotech Autoplate 4000. Test tubes containing TSB were vortexed before samples were withdrawn for spiroplating. Samples from test tubes containing FTM were not vortexed and taken from the bottom of the tube below the pink layer for spiroplating. Samples were manually loaded from the Eppendorf tubes into the machine stylus. The stylus then plated the samples, and was put through a washing phase to be washed with bleach and sterilized water.

Ofloxacin Formulations

Ofloxacin was incorporated into the microemulsion systems. 4 mM solutions of Ofloxacin were prepared by weighing an appropriate amount of the solid in a 5 mL volumetric flask. The solid Ofloxacin was dissolved by adding approximately 4.5 mL RO water to the flask and placed in an incubator overnight at 30°C to increase the rate of dissolution. Once completely dissolved, the solution was made up to the 5mL mark with RO water. Aliquots of the 1:5, 1:10, and 1:15 DOSS/EO and DOSS/PMP mixtures were weighed on an analytical balance. The DOSS/oil mixtures were then titrated with the Ofloxacin solution following the same procedure as the formulation of microemulsions with RO water and buffers. All experiments were done in triplicate. Maximum percent Ofloxacin solution incorporated was compared to maximum percent RO water incorporation for each formulation.

Dissolution Studies

Methylene blue was used to study the release in microemulsion systems. A calibration curve was first prepared using a 0.02 M stock solution of methylene blue. Five methylene blue dilutions were prepared in test tubes containing 2.5, 5, 7.5, 10, and 15 µL of the stock dye solution. Then, to each of the tubes, a sufficient volume of RO water was added to achieve a final volume of 4 mL. The tubes were vortexed, and the absorbance was then determined for each of the test tubes at 294 nm using a Spectronic 20 Genesys Spectrophotometer (Spectronic Instruments) with RO water as a blank. The absorbance was plotted versus concentration of dye (µmol/mL) to obtain a calibration curve.

A 1M methylene blue solution was prepared for the microemulsion formulations by dissolving 3.739 g of methylene blue powder in 10mL of RO water. An aliquot of the methylene blue solution was added to an appropriate quantity of 1:10 DOSS/EO mixture taken in a test tube,

and the tube was vortexed. The sample was transferred into a Spectra/Por® Biotech Cellulose Ester Dialysis Membrane, molecular weight cut-off of 100,000Da (Spectrum Laboratories, Rancho Dominguez, CA), clamped on the ends to prevent microemulsion spillage. The filled dialysis membrane was submerged in 1 L of RO water containing a magnetic stirrer and kept in a water bath at 37°C. Samples of 4 mL were withdrawn from the RO water, transferred to test tubes, and replaced with fresh RO water at various time points. The absorbance of the samples was determined at 294 nm using a Spectronic 20 Genesys. The concentration of methylene blue (μmol/mL) was determined from a previously prepared calibration curve. Methylene blue concentration was plotted versus time to create a release profile. Experiments were done triplicate.

RESULTS AND DISCUSSION

Formulation of Microemulsions

Surfactant/oil mixtures were prepared (w/w) in ratios of 1:5, 1:10, and 1:15 using five oils, which include EO, PMP, GTCC, Labrafac Lipophile, and oleic acid. Water was added drop-wise to the surfactant/oil mixtures using the titration method and visually inspected to determine the amount of water able to be incorporated into each formulation. Phase diagrams were constructed to visualize the microemulsion forming regions. Systems containing GTCC, Labrafac, and Oleic acid as the oil showed fairly low incorporation of water to form visually clear microemulsion systems. Because of this, they were not selected for further investigation. The PMP and EO containing systems showed the most potential for microemulsion formation, due to their ability to emulsify a sufficient amount of water (>10% for over half of all surfactant/oil ratios with EO and PMP) while still remaining as a visually clear and homogenous microemulsion. Because of this, these two surfactant/oil systems were chosen for further characterization and drug incorporation.

Knowledge of the hydrophobicity or polarity of the oil can help in determining its microemulsion-forming abilities. Oils can be assigned an equivalent alkane carbon number, or the oil's polarity can be measured by quantifying its miscibility with water alone. When mixing the oil and the water, relative volumes added may change if the oil in itself is partly miscible with water. If the oil contains any lipophilic surface-active components, an emulsion may result, even without the addition of a surfactant. EO may be an example of this. Crodamol PMP is not only considered an oil, but it is a good solvent as well. In some formulations, Crodamol PMP is used in part as an emulsifier. Its ability to incorporate such large amounts of water in the microemulsions could be due to the fact that along with adding a surfactant to the system, the oil itself had inherent emulsifying properties. Oleic acid is also used in combination with other components as an emulsifying agent as well. This may explain its ability to incorporate more water than the two triglyceride formulations, GTCC and Labrafac, which do not contain any lipophilic surface-active components. Depending on the size of the oil, relative to the hydrophobic chain of the surfactant, the oil may penetrate into the surfactant tails of the interfacial monolayer. Smaller oil molecules would position themselves among the surfactant tails, which would increase the effective surfactant hydrophobe volume, effecting the CPP.

Effect of Surfactant Concentration on Microemulsion Formation

Surfactant/oil systems of various concentrations were prepared using all the oils tested earlier. The ratio of DOSS/oil systems used in this study include 1:4, 1:5, 1:7, 1:10, and 1:15. All surfactant/oil systems were titrated with water to determine the microemulsion forming regoin. It represents the maximum percent of water incorporated in to different surfactant/oil systems forming a clear and homogenous microemulsion. Ethyl oleate was the only system in which a visual turbidity appeared while being titrated with water. After some equilibration time, this turbidity would disappear and a clear microemulsion was observed. During titration with water, as the concentration of water came close to the border, requirement for an equilibration time to observe a clear microemulsion decreased. As with all other formulations, once the maximum amount of water that could be emulsified was added, the sample appeared turbid, and did not clear after 24 hours of equilibration. Because of the observed turbidity at Wo's before the microemulsion border was reached, EO microemulsions were prepared 24 hours in advance before any other tests were performed. This ensured that an equilibrated system was subjected to subsequent characterizations.

Buffer and pH Effects

The effect of pH on the amount of water emulsifies into a microemulsion was investigated using various buffers. DOSS/EO and DOSS/PMP in a ratio of 1:10 were titrated with buffers of varying pH, and the maximum amount of buffer incorporated into a clear microemulsion was determined.

As a general trend, the surfactant/oil mixtures emulsified less buffer than RO water. 1:10 DOSS/EO was able to emulsify 6.2% RO water, and the same ratio of DOSS/PMP could emulsify 14.3% RO water. However, the DOSS/EO mixture was able to incorporate almost 1% more buffer than RO water when titrated with pH 4 acetate. The amount of pH 5 acetate buffer able to be emulsified was almost equal to that of RO water. In DOSS/EO mixtures, buffers from pH 6 to pH 11 significantly reduced the amount of water incorporated as a clear microemulsion.

The total amount of aqueous buffer incorporated into a clear microemulsion in DOSS/PMP mixture was less than 6.5% for buffers of pH 4 to 11. DOSS being an ionic

surfactant may possibly interact with salts present in the buffers reducing its ability to properly orient in the interfacial region. The interfacial film may be increasing in disorder, as can sometimes be seen with co-surfactants, due to the presence of the buffer salts resulting in a lessened ability to emulsify water. This phenomenon may be attributed to the reduced amount of water emulsified into a clear microemulsion in these surfactant/oil mixtures.

Polarized Light Microscopy

Microemulsions are optically isotropic colloidal dispersions that exhibit no birefringence under polarized light, so samples that exhibit no birefringence suggests that they are microemulsions. DOSS/EO and DOSS/PMP in a ratio of 1:15 (surfactant:oil) were used for the polarized light microscopy studies. Compositions that formed visually clear and stable microemulsion represented within the microemulsion forming region in the ternary phase diagram were selected in this study. Similarly, compositions at the boundary region of microemulsion area and beyond the microemulsion region were also used in the study. Microemulsions possessing Wo values of 25, 30 and 195 were prepared in DOSS/EO mixtures. Sample with Wo = 25 exhibited no birefringence under polarized light, indicating that it was a microemulsion.

Microscope pictures of Wo = 30 with and without polarized lens shows limited structural delineations indicating that the formulations contained structures other than droplets. These formulations represent compositions that are leaving the microemulsion forming region in the ternary phase diagram. Contrasting the earlier result was the Wo = 195 sample that exhibited a droplet structure visible under normal and polarized light. This indicates formation of a macroemulsion at this composition.

Microemulsions prepared in DOSS/PMP mixtures incorporated larger quantity of water when compared to DOSS/EO mixtures. Microemulsions possessing Wo values of 25, 75, and 95 were prepared, representing samples that were within the microemulsion region, at the microemulsion border, and beyond the microemulsion region respectively. When observed under the microscope, DOSS/PMP samples with Wo values of 25 and 75 showed no birefringence through a polarizing filter and represent microemulsions. The formulation with a Wo of 95 exhibited bicontinuous structures under the polarized lens, indicating that it was beyond the optically isotropic microemulsion region. Air bubbles in the sample that were trapped under the slipcover are visible, and debris that was present.

Conductivity

Electrical conductivity testing was done using DOSS/EO and DOSS/PMP mixtures in ratios of 1:5, 1:10 and 1:15. The amount of water present in the formulations was modulated by varying the Wo value. The observed conductivity values were then plotted as a function of Wo. All formulations demonstrated composition dependent changes in conductivity. Conductivity values of 0.011 μS/cm, 0.033 μS/cm, and 0.122 μS/cm were observed at small Wo's. This indicates that oil, which is known to have low conductivity, is the continuous phase and water droplets surrounded by surfactant constitute the internal phase. A percolation phenomenon is often observed for water-in-oil (w/o) emulsions where the electrical conductivity steeply rises above a certain threshold as the water concentration increases, as if water was becoming the continuous phase.

A percolation like phenomenon was observed in microemulsions prepared in 1:5 DOSS/EO mixtures as demonstrated by large fluctuations in conductivity values as Wo value changed from 20 to 25. In this surfactant/oil ratio, a Wo value of 20 still represents a microemulsion formulation, but a Wo of 25 is beyond the microemulsion region. In the 1:5 DOSS/EO system, a formulation with Wo value of 25 appeared turbid, further demonstrating that the system had crossed the water-in-oil microemulsion threshold. These conductivity results also correlate with the data obtained from phase diagrams constructed during the microemulsion formulation.

In 1:10 DOSS/EO mixture, a significant increase in conductivity was recorded when Wo changed from 20 (6.7% w/v water) to 25 (8.3% w/v water). The sample containing 6.7% (w/v) water is still in microemulsion region, but 8.3% (w/v) water is outside of microemulsion region. Visible cloudiness was observed in Wo of 25 and 30 formulations indicating that they are not microemulsions. The Wo 30 formulation prepared in 1:10 DOSS/EO mixture separated into constituent phases while measurements were performed.

In 1:5 DOSS/EO mixture, a change in the Wo value from 2.5 to 25 did not produce significant changes in the measured conductivity values. This indicates that all mixtures with Wo's from 2.5 to 25 are within the microemulsion region. None of the samples showed visible cloudiness or turbidity, further validating the determination that they were all within the microemulsion region.

DOSS/PMP formulations are able to emulsify a greater amount (29.7%) of water as a water-in-oil microemulsion than DOSS/EO formulations, as was seen in the formulation phase diagrams. Because of this, Wo values of up to 100 could be tested for electrical conductivity. A sudden increase in conductivity along with fluctuating values were noted when the Wo reached 95 in a 1:15 DOSS/PMP mixture. The dramatic increase in conductivity correlated with the observation of visual cloudiness in the sample. The percolation phenomenon has been noticed and studied by many. W/o microemulsions systems, such as those seen in this research, especially have exhibited this percolation behavior throughout a variety of microemulsion studies. The formation of a bicontinuous state has been suggested to aid percolation. Feldman et al. and Mukhopadhyay et al. suggest that the conductivity increase seen in percolation is due to the

counterions moving throughout the bicontinuous water channels resulting in an increase in conductivity.

Rheology

W/o microemulsions are known to exhibit Newtonian flow properties as well as relatively low viscosities. DOSS/EO microemulsion samples with Wo values of 2.5, 5, and 7, exhibited Newtonian flow properties. As shear rate varied, viscosity remained relatively constant. This consistent with rheological behavior reported in other microemulsions.

Rheology

W/o microemulsions are known to exhibit Newtonian flow properties as well as relatively low viscosities. DOSS/EO microemulsion samples with Wo values of 2.5, 5, and 7, exhibited Newtonian flow properties. As shear rate varied, viscosity remained relatively constant. This consistent with rheological behavior reported in other microemulsions. Microemulsion samples showed relatively low viscosities, close to that of the oil used, ethyl oleate. Viscosity slightly increased with increasing water concentration, which is similar results were seen with the DOSS/PMP microemulsion systems. Particle sizes ranged from 6.7 to 26.6 nm in diameter. Larger Wo values were tested for DOSS/PMP formulations since DOSS/PMP mixture could emulsify a larger percent of water.

A general trend that was observed for both DOSS/EO and DOSS/PMP systems was that the particle size increased with an increase in surfactant concentration at a particular Wo value. This is most likely due to the fact that with more surfactant in the formulation, a larger volume of the internal phase will be emulsified, ultimately resulting in larger droplets.

Development of Sterilization Method

An aseptic filtration method of sterilization that can be universally applied to similar microemulsion formulations was developed using DOSS/EO microemulsions that were formulated in this research project. Microemulsions were prepared using 1:5 DOSS/EO and sterile water for injection to achieve Wo's of 2.5, 10 and 15. Microemulsions were withdrawn into a sterile syringe and passed through a 0.22 μm filter directly onto an MH Agar plate in a laminar hood. After allowing the formulation to absorb into the agar, the plates were incubated and checked for microbial growth after 24 hours. Growth is generally indicated by the cloudy appearance of the agar after incubation. All three samples showed white cloudiness on the plates after 24 hours incubation. Upon visual inspection and dissection of the agar, it was determined that the cloudiness was within the agar and not on the surface, where colonies are generally found. The water used in the formulations was commercial sterile water for injection and was therefore ruled out as a possible source of the cloudiness.

Bacteria cannot grow in oil, because water is necessary for their growth. It may be possible, though, for microbes to be dormant within oil, so EO and the DOSS/EO mixture were further examined. To determine the source of the cloudiness, EO and the DOSS/EO mixture were plated on MH Agar plates. Filtered and unfiltered EO and DOSS/EO mixture were plated on MH agar plates. Four agar plates containing the test liquid were incubated for 24 hours and checked for signs of microbial growth. The plates containing the unfiltered and filtered EO showed no cloudiness. However, the plates containing both filtered and unfiltered DOSS/EO mixtures displayed white cloudiness similar to that seen with the microemulsion samples. This led to the conclusion that DOSS is the source of the cloudiness.

The white cloudiness in the agar plates that contained DOSS could be the result of DOSS precipitation since it is of itself a white, waxy substance that is not very soluble in water, which is a main component of MH agar. As the microemulsions permeate into the agar, it is possible that the emulsion breaks apart allowing the white, waxy DOSS to be visible. To further examine the possibility that the cloudiness was caused by the formulation itself and was not due to microbial growth, filtered microemulsion samples and sections of the cloudy MH agar were investigated. Small squares of the cloudy MH agar from the microemulsion samples and from the DOSS/EO samples were excised and placed into individual test tubes that contained MH broth. Filtered microemulsions were directly introduced into test tubes containing MH broth as well, which turned cloudy upon vortexing. After 24 hours of incubation, samples were taken from each of the test tubes, applied to slides, and gram-stained to determine the presence of bacteria. When all slides were observed under a microscope after gram staining, tiny irregularly shaped objects were visible. These were significantly smaller than bacteria, and were not the shapes of bacteria that are usually seen from gram-staining [30]. This experiment confirmed the identity of the tiny irregularly shaped objects as precipitated DOSS.

Validation of Sterilization Method

Formulations identical to those used in sterility testing were used in the validation of sterility. Samples were analyzed using a direct inoculation technique as well as filtration, utilizing two different broths according to USP standards. Fluid thioglycollate medium (FTM) is used for the growth and detection of anaerobic bacteria. Soybean casein digest medium or tryptic soy broth (TSB) is used for the detection of fungi and aerobes. Samples were filtered through a 0.22 μm filter system under aseptic conditions. As per USP standards, the sample was passed through the filter, and the filter was rinsed with sterile water. The filter was then cut into three pieces and placed in test tubes that contained TSB (stored at 20°C), TSB (stored at 35°C), or FTM (stored at 35°C). The samples stored at 35°C mimicked temperatures close to body

temperature which promote the growth of aerobic and anaerobic bacteria. Samples stored at room temperature (20°C) were used for the detection of fungi. All filtration test tubes except for the first positive control remained clear throughout the 14 days of the study. The same samples were directly inoculated into test tubes of media. After sitting, the direct innoculation tubes that contained the microemulsion samples separated into three layers. Before samples were taken to plate on the blood agar, the TSB test tubes were vortexed to ensure uniformity. The negative control and second positive control were the only tubes that remained clear throughout the entire 14 day testing period. All others, upon vortexing, were uniformly turbid. The USP indicates that if the material being tested for sterility renders the medium turbid when directly inoculated, the samples are to be diluted in the medium. This cannot be done for the microemulsion formulations being tested. As was seen when formulation and phase diagram studies were performed, once the microemulsions reached a certain water concentration, the formulations appeared turbid. Since the media used were water based, dilution of the samples would only add more water to the systems and increase their turbidity. Since turbidity of the test tubes was not able to be used as the detection of microbes as per the USP standards, another method was employed.

To detect the presence of microbes, samples from the test tubes were taken and transferred onto blood agar plates. Samples were taken from the filtration and direct inoculation test tubes on days 0, 7, and 14 for plating. After 24 hours incubation at 35°C, the plates could then be inspected for microbial growth. Day 0 showed slight growth only on 4 of the 6 first positive control plates. All samples plated on Days 7 and 14 showed no growth except for the first positive control. Negative control plates were always absent of microbial growth. Only the first positive control showed growth on the plates, as well as turbidity in the test tubes. The second positive control was most likely too low of a bacteria concentration, and therefore did not show any growth. Plates with the microemulsion samples did not show microbial growth. Direct inoculation microemulsion plates showed a transparent ring in the blood agar where the microemulsion sample was most concentrated. It appeared that the microemulsion samples hemolyzed the red blood cells in the agar plates. Representative pictures of the blood agar plates.

Cell Toxicity Studies

A neutral red assay was performed on the cells that had been exposed to microemulsions of varying DOSS/EO concentrations and Wo values. In this assay, the dye is absorbed by live cells and stains the mitochondria within. Cells that are not living cannot retain the dye when washed. After being exposed to dye and then washed, all color that remains will be due only to the living cells that absorbed and retained the dye. Absorbance readings were taken for each well. To determine cell toxicity, the absorbance of the wells that were exposed to microemulsion samples were compared to a control well which contained cells without a microemulsion treatment. Treatments were considered to be non-toxic if the absorbance from the cells treated with a microemulsion was similar to or the same as the absorbance from the control well. Along with the neutral red assay, a Bradford protein assay was performed to standardize the absorbance readings. The assay stains proteins so the number of cells present in each well could be determined. This allows you to relate the total number of cells in the well to the absorbance of the live cells. From this, cell toxicity can ultimately be determined when comparing the treated wells to the control well. When compared to the control cells, the only DOSS/EO microemulsion samples that showed toxicity were samples prepared with 1:15 DOSS/EO mixture having Wo's of 10 and 15.

As a general rule, the higher the surfactant concentration, the more water can be incorporated into the system. DOSS/EO mixture in the highest surfactant ratio of 1:4 was able to emulsify the largest amount of water (13.7%) while the lowest surfactant concentration of 1:15 emulsified the lowest (4.0%). Toxicity was seen only in 1:15 DOSS/EO mixtures with Wo values of 10 and 15. The media added in this experiment is water-based. Because of this, when the media is added to the wells, it ultimately results in a net increase in the amount of water present in the internal phase of the formulation. Since the formulations tested were w/o microemulsions the addition of more water to the systems does not constitute a simple dilution. As stated earlier, the 1:15 DOSS/EO mixture emulsifies the least amount of water of all DOSS/EO mixtures. The 1:15 DOSS/EO microemulsion samples with Wo values of 10 and 15 already contained close to the maximum amount of water for that system. When the aqueous media was added over the layer of these formulations in the wells a breach of the percolation threshold can occur. This results in turbidity and increased viscosity of the original microemulsion. Because these two samples were no longer transparent microemulsions after the media was added, and the systems were more viscous, the media may not sufficiently reach the cells in order to keep them viable. All other formulations and dilutions tested were able to incorporate more water, which allowed them to remain as intact microemulsions even upon addition of the media.

Ofloxacin Formulations

A solution of Ofloxacin was titrated with DOSS/EO and DOSS/PMP concentrations 1:5, 1:10, and 1:15. Similar trends were seen with the incorporation of Ofloxacin solution as were seen with RO water. 1:5 DOSS/oil mixtures emulsified the most Ofloxacin solution followed by 1:10, and then 1:15 with the least. Also, DOSS/PMP mixture emulsified a larger amount of Ofloxacin solution than DOSS/EO mixture. The percent

w/w Ofloxacin solution emulsified in the respective DOSS/oil mixtures. A slight increase in the quantity of Ofloxacin solution capable of incorporation in the DOSS/EO and DOSS/PMP mixtures was observed. One possibility for this may be that the drug is slightly surface active in itself. A large number of drug molecules have been shown to be surface active.

Dissolution Studies

The calibration curve obtained for methylene blue standard solutions. The equation for the line was calculated to be $y = 0.0251 + 53.9272x$ with an R^2. Absorbances obtained from the release study were substituted into the equation from the calibration curve to determine the concentration of methylene blue present. This was plotted versus time to determine a dye release profile. Methylene blue release was recorded over a 72-hour time period. Release seemed to begin to plateau between 48 and 72 hours. At 72 hours in the experiment, all three trials released approximately 0.009 μmol/mL of methylene blue. The dissolution profile demonstrates a controlled release pattern for the model drug.

Table 1. Percent (%) water incorporation in each of the oils for samples (in triplicate)

Oil	1:4	1:5	1:7	1:10	1:15
EO	13.7 ± 0.3	11.1 ± 0.3	8.5 ± 0.5	6.2 ± 0.7	4.0 ± 0.6
PMP	29.7 ± 0.4	23.9 ± 1.3	20.6 ± 0.2	14.3 ± 0.9	11.7 ± 0.3
GTCC	n/a	2.6 ± 0.3	n/a	1.6 ± 0.6	0.9 ± 0.1
Labrafac	n/a	2.3 ± 0.5	n/a	1.1 ± 0.4	0.9 ± 0.1
Oleic Acid	n/a	5.9 ± 0.4	n/a	2.7 ± 0.3	2.1 ± 0.6

Table 2. Summary of results on blood agar plates indicating the presence (+) or absence (-) of growth

Day 0		Direct			Filtration	
	FTM	TSB	TSB 20	FTM	TSB	TSB 20
Negative Control	-	-	-	-	-	-
First Positive Control	+	+	+	-	-	+
Second Positive Control	-	-	-	-	-	-
Wo = 2.5	-	-	-	-	-	-
Wo = 10	-	-	-	-	-	-
Wo = 15	-	-	-	-	-	-
Day 7						
Negative Control	-	-	-	-	-	-
First Positive Control	+	+	+	+	+	+
Second Positive Control	-	-	-	-	-	-
Wo = 2.5	-	-	-	-	-	-
Wo = 10	-	-	-	-	-	-
Wo = 15	-	-	-	-	-	-
Day 14						
Negative Control	-	-	-	-	-	-
First Positive Control	+	+	+	+	+	+
Second Positive Control	-	-	-	-	-	-
Wo = 2.5	-	-	-	-	-	-
Wo = 10	-	-	-	-	-	-
Wo = 15	-	-	-	-	-	-

Table 3. Summary of results showing absence (-) and presence (T) of toxicity with 1:5,1:7, 1:10, and 1:15 DOSS/EO microemulsions of varying Wo's

DOSS/EO Ratio			Wo Values	
	2.5	5	10	15
1:5	-	-	-	-
1:7	-	-	-	-
1:10	-	-	-	-
1:15	-	-	T	T

Table 4. Percent (%) Ofloxacin solution emulsified by DOSS/EO and DOSS/PMPmixtures of 1:5, 1:10, and 1:15 (in triplicate)

	1:5	1:10	1:15
EO	12.99 ± 0.38	7.20 ± 0.17	4.18 ± 0.07
PMP	29.40 ± 0.06	19.55 ± 0.17	14.91 ± 0.42

Fig: 2. Ternary phase diagram representing the microemulsion-forming compositionsin DOSS/PMP/water systems

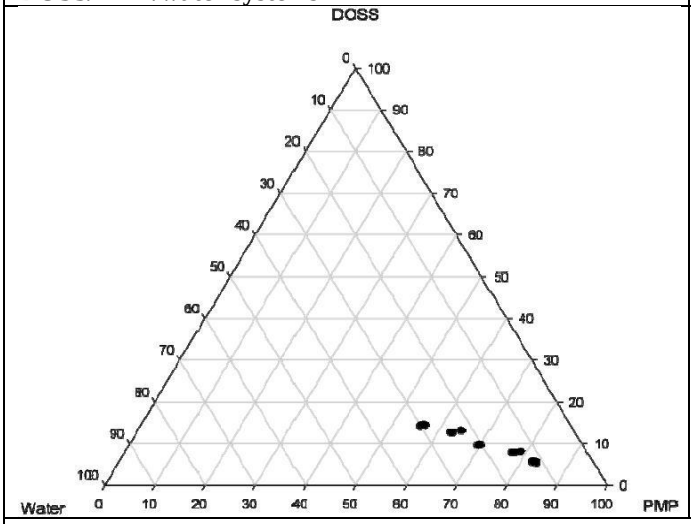


Fig: 3. Dependence of pH on percent water incorporated as a clear microemulsion in1:5 DOSS/EO (□) and DOSS/PMP (◦) mixtures

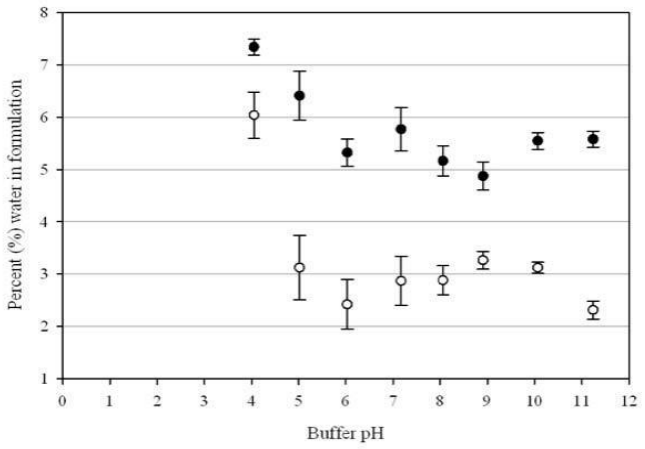
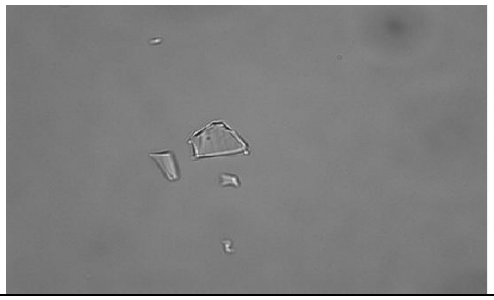


Fig: 4. Pictures of DOSS/EO Wo=25 without (a.) and with (b.) polarizing filter

a.



b.

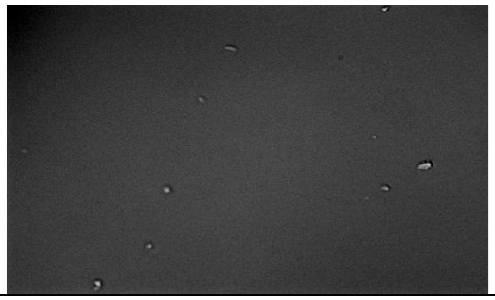


Fig: 5. Conductivities for microemulsions at various Wo's for DOSS/EO ratios 1:5(□), 1:10 (◦) and 1:15 (▼)

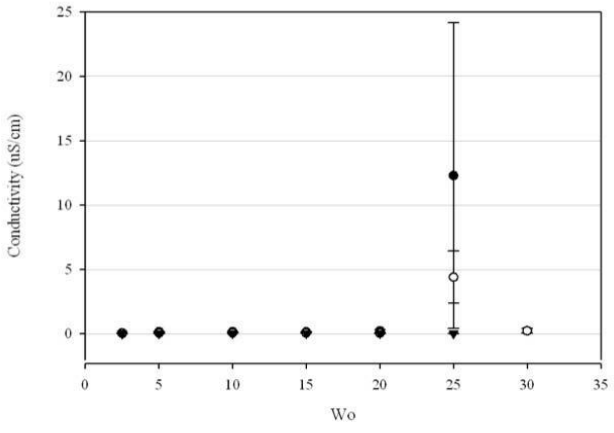


Fig: 6. **Figure 4.14** Conductivities for microemulsions from Wo = 2.5 to Wo = 20 for DOSS/EOratios 1:5 (□), 1:10 (◦) and 1:15 (▼)

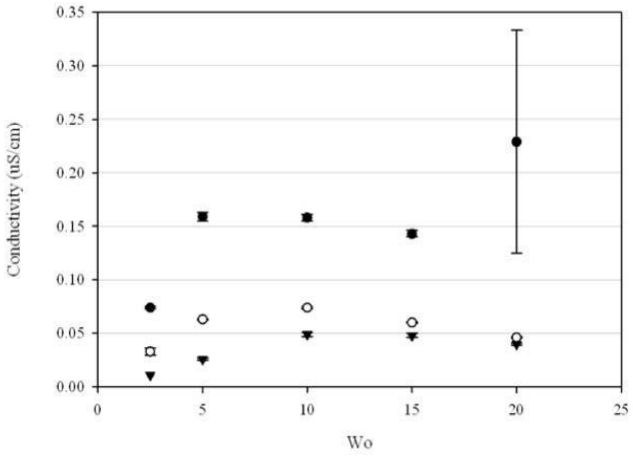


Fig: 7. Graph showing viscosity of DOSS/EO microemulsions with Wo's of 2.5, 5,and 7.5 at varying shear rates

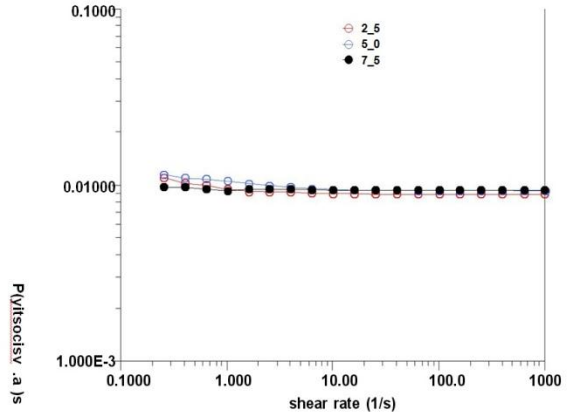


Fig: 8. Graph of mean volume weighted diameter for microemulsions

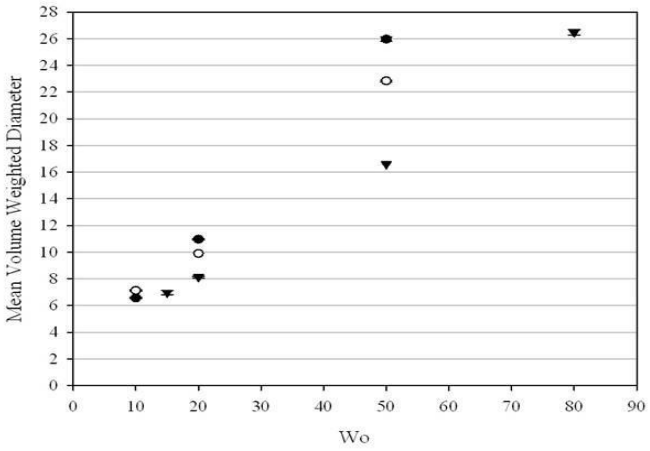


Fig: 9. Representative picture of test tubes containing filters with the negativecontrol (a), first positive control (b), second positive control (c), Wo = 2.5 (d), Wo = 10 (e), and Wo = 15 (f). Test tubes remained visually the same over the 14 day testing period

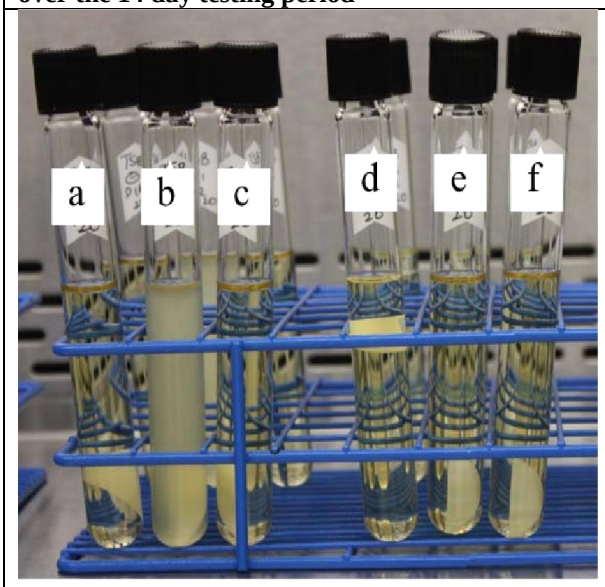
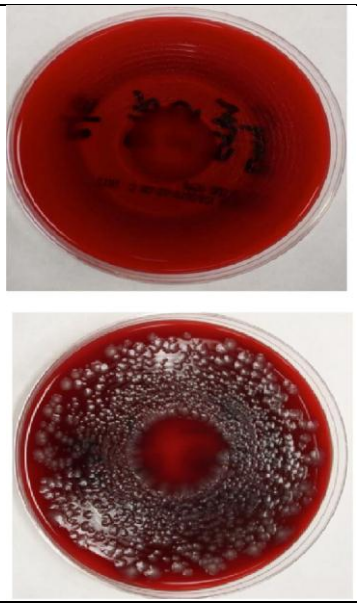


Fig: 10. Representative pictures of blood agar plates



CONCLUSIONS

Microemulsions were formulated with water, diocytl sodium sulfosuccinate, and two oils, EO and PMP. Characterization through conductivity, rheology, polarizing light microscopy, and DLS helped to define the clear, microemulsion forming regions. Microemulsions exhibited Newtonian flow and relatively low viscosities slightly higher than the viscosity of the oil itself. No birefringence was observed in microemulsion-forming compositions. Conductivities were low indicating that the systems were water-in-oil microemulsions. Once a certain concentration of water was added, a percolation phenomenon was observed where the conductivity drastically increased, indicating the water was becoming the continuous phase of the system. DLS measurements indicated that particle sizes were below 30nm for all Wo values in DOSS/EO and DOSS/PMP microemulsion systems. When compared to RO water, buffer and pH appeared to have an impact of the percent water incorporated in microemulsions. In general, a smaller percentage of buffer was able to be incorporated into both DOSS/EO and DOSS/PMP systems than RO water. An Ofloxacin solution was successfully incorporated into mixtures of DOSS/EO and DOSS/PMP. The percentage of Ofloxacin solution able to be emulsified

by DOSS/EO and DOSS/PMP mixtures was slightly higher than RO water. The percent RO water and percent Ofloxacin solution able to be incorporated into the DOSS/EO and DOSS/PMP microemulsions indicates that these formulations show potential for drug delivery applications. An aseptic filtration method of sterilization that can be universally applied to similar microemulsion formulations was successfully developed and validated using DOSS/EO microemulsion systems as a model. The sterilization of the microemulsions shows potential to be used in ophthalmic formulations.

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