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A Novel Biocompatible, Membrane-Interactive Delivery System for Administration of an Antimicrobial to Treat MRSA

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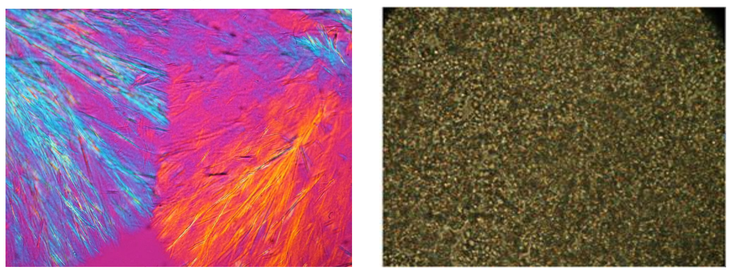
Treating MRSA: A New Approach

In the treatment of ocular microbial disease, bacterial resistance, a result of mutations that become established after successive generations of antibiotic exposure, is a recurring phenomenon that has rendered existing antimicrobial treatments are rendered ineffective by emergent multi-drug resistant species. This is a subject of great importance in ophthalmology: methicillin-resistant *S. aureus* (MRSA) has been frequently found in bacterial isolates obtained from cataract surgery patients and from those who suffer from diseases such as ocular keratitis and blepharitis. Based on world-wide surveillance studies on drug susceptibility to MRSA, Moxifloxacin is an excellent drug candidate for the treatment of drug-resistant infections and currently exist in commercially available products (Moxexa™, Vigamox™), which are both solutions of moxifloxacin hydrochloride. We have hypothesized that drug absorption can be improved by formulation enhancements that enable high bioavailability. To that end, we have developed **OcuSurf™**, a drug delivery system composed of a biphasic drug-containing nanoparticle emulsion that can be administered to ocular tissues, both for front-of-the-eye and back-of-the-eye indications. It is liquid crystalline and is capable of encapsulating high concentrations of insoluble drug compounds. Herein, we present data regarding the development of OcuSurf-Moxifloxacin (OS-Mox), comparing two formulations. Formulation 1 contains Moxifloxacin Hydrochloride, which is unsuitable for membrane penetration or for encapsulation. Formulation 2 contains the freebase form. We report higher log P, *in vitro* release and *ex vivo* corneal penetration of moxifloxacin free base as delivered by moxifloxacin. Our OS-Mox formulation is unique in its ability to encapsulate and deliver the freebase form to the corneal tissue where it can be absorbed.

OCUSURF™ DELIVERY SYSTEM IS LIQUID CRYSTALLINE



OcuSurf™-MOX-FB Drug Phase

OcuSurf ™ final dispersion

Figure 1: OcuSurf MOX-FB formulation examined under crossed polarizers, on an Olympus BX51P Polarizing Light Microscope, at up to 1000X.

OcuSurf™-Moxifloxacin

- *Platform Technology, enabled for multiple small molecule drugs.*
- *Liquid crystalline, ordered structures, characterized by polarized light microscopy. This enables sustained release due to entrapment of molecules in the nanostructures.*
- *Sustained release enabled by following mechanisms:*
  LC state, fast absorption into cornea, membrane interaction.
- *High bioavailability enabled by presence of dissolved drug in nanostructure cores.*
- *High permeability enabled by the use of membrane-interactive permeation enhancers.*
- *Scaleable*

OcuSurf-Moxifloxacin: Characterization and Results

PREPARATION

A biphasic nano-dispersion was prepared using both hydrophobic and hydrophilic GRAS excipients. In Formulation 1, Moxifloxacin Hydrochloride was dissolved in the hydrophilic phase. In Formulation 2, Moxifloxacin Freebase was dissolved in the hydrophobic phase. The cores are suspended in an aqueous hydrophilic continuous phase which keeps the cores dispersed and suspended. The dispersed cores form liquid crystalline ordered structures—that act as one of the mechanisms that enable sustained release. Process techniques enable control of particle size. In addition to moxifloxacin, we have encapsulated multiple drugs in OcuSurf™, demonstrating its platform utility.

MOXIFLOXACIN FREEBASE EXTRACTS EFFICIENTLY IN LIPIDS

LogP	[MOX]Oct. (mg/g)	[MOX]Aq. (mg/g)	O/A Ratio	LogP
Mox FB	1.02	0.06	17.68	1.25
Mox-HCl	0.12	0.50	0.25	-0.60

Table 1: A LogP partition coefficient test was performed by suspending the freebase and the hydrochloride forms of moxifloxacin. The octanol and aqueous fractions were assayed at λ=295nm after overnight incubation at 37°C. The log of the octanol-to-aqueous concentration ratio is a measure of the respective APIs' hydro- and lipophilicity. The freebase is far more lipophilic, indicating that it would extract/absorb more efficiently into membranes. .

MOXIFLOXACIN IS STABLE AND SURVIVES THE OCUSURF™ PROCESS

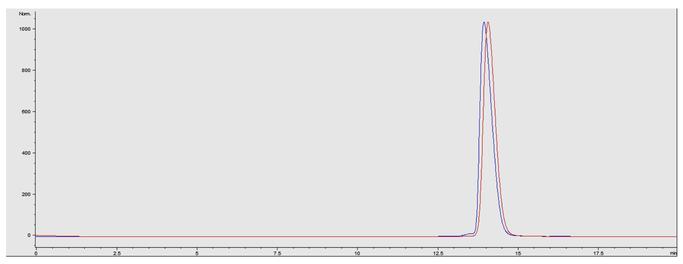


Figure 2: OcuSurf-Mox-FB and -HCl formulation samples were assayed by HPLC, and it was found that >99% of the drug content had survived the encapsulation and emulsification process. Samples were 1mg/g w/w API and analyzed at λ=295nm. In the above chromatogram, the red peak is the freebase and the blue peak is the hydrochloride.

IN VITRO RELEASE OF DOSAGE FORMS

Method in Development

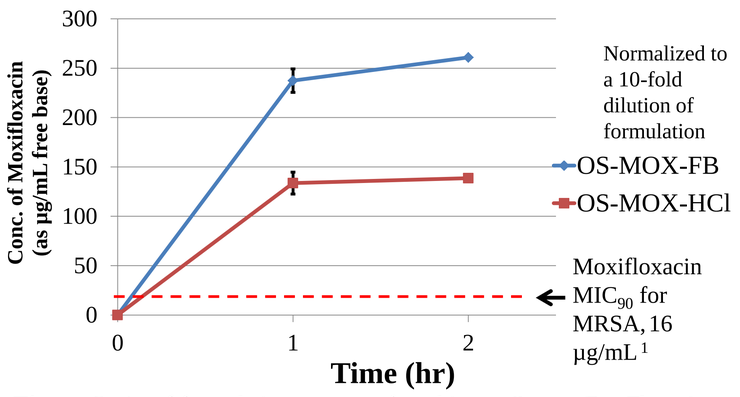


Figure 3: 1g of formulation was transferred into a Spectra/Por Float-A-Lyzer G2 Dialysis Device, which was then placed into a 50 mL tube containing 30 grams of 1% HPβCD in phosphate buffer pH 7.4, 37ºC.. The results demonstrate that the API loaded within OcuSurf is quickly released upon application. For reference, the minimum inhibitory concentration of Moxifloxacin for MRSA is marked.

OCUSURF™ PARTICLE SIZE IS REPRODUCIBLE AND STABLE

Drug Type	Formulation	Particle Size (D50/D90) T0 (µm)
Steroid	OcuSurf-FP, 0.1% FP*	0.151/0.259
Steroid	OcuSurf-FP, 0.2% FP*	0.159/0.281
Steroid	OcuSurf-LE, 0.1% LE*	0.159/0.356
Antimicrobial	**OcuSurf-MOX-HCl (Moxifloxacin.HCl)**	0.168/0.264
Antimicrobial	**OcuSurf-MOX-FB (Moxifloxacin free base)**	0.174/0.286

Table 2: The particle size of the nanocores is consistent across batches and across drug type, with a D50 of 150-170nm, and a D90 of 250-290nm. The adjustment of process conditions allows for the adjustment of this size distribution. In addition, an 8-month measurement was taken of the first batch. The D50/D90 size was 0.152/0.274, indicating long-term stability. PSD measurement was performed using a Horiba LA-950V2 laser diffraction apparatus.

ENCAPSULATION IN OCUSURF™ FACILITATES CORNEAL PERMEATION

	OS-MOX-HCl (n=3)		OS-MOX-FB (n=3)	
Time (hours)	**Ave. µg Mox-HCl permeated**	**St. Dev.**	**Ave. µg Mox-FB permeated**	**St. Dev.**
1 h	0.1	n/a	0.1	n/a
3 h	**0.9**	0.9	**0.9**	0.5
5 h	**3.1**	2.9	**4.1**	1.5
7 h	**6.2**	5.2	**9.3**	3.0
24 h	**38.1**	23.3	**73.4**	5.4
Cornea	**10.9**	1.9	**15.3**	3.3
Total (Cornea +24h perm.)	**49.0**	25.1	**88.8**	2.1

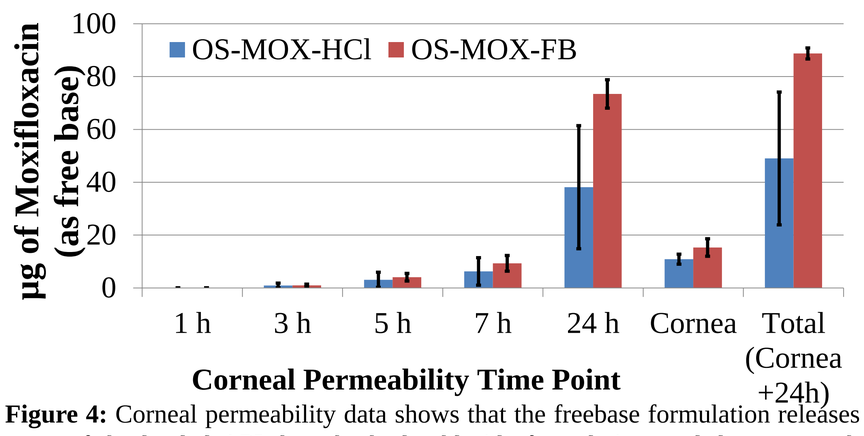


Figure 4: Corneal permeability data shows that the freebase formulation releases more of the loaded API than the hydrochloride formulation, and does so much more consistently. This result demonstrates that OcuSurf facilitates the passage of moxifloxacin across the corneal membrane. The hydrochloride formulation is able to permeate, but without complete encapsulation, the permeability is more variable by 24 hours. Permeability sampling was performed on a Franz diffusion cell apparatus at 37°C. The permeation buffer was 1% cyclodextrin in pH 7.4 phosphate buffer. Calf corneas were used for this experiment (obtained from Research 87). Drug was extracted by the cornea by homogenizing a corneal section in permeation buffer, centrifuging the slurry, and assaying the supernatant. Permeability samples were assayed by HPLC at λ=295nm

Conclusions and Next Steps

- The OcuSurf™ platform enables a tissue-permeating delivery system for the antimicrobial Moxifloxacin.
- Moxifloxacin, encapsulated either as the hydrochloride salt or as the free base, is rapidly bioavailable to deliver an effective dose and thus, an excellent 505b2 candidate for development.
- Moxifloxacin free base encapsulated in the hydrophobic nanocores of OcuSurf™ provides consistent permeation into and through corneal tissue.
- **Drug permeated into tissue can also act as depot for sustained release.**
- Next steps will include additional corneal permeability studies, optimization of the in vitro release model, formulation optimization, and MIC testing of the OcuSurf™-MOX drug products.

Acknowledgements

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[1] Asbell PA, Sanfilippo CM, Pillar CM, DeCory HH, Sahm DF, and Morris TW. Antibiotic Resistance Among Ocular Pathogens in the United States: Five-Year Results From the Antibiotic Resistance Monitoring in Ocular Microorganisms (ARMOR) Surveillance Study. JAMA Ophthalmol 2015;133(12):1445-54.

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