Creation and Release Profiles of Novel Biodegradable Ointments for Lesion Based and Whole Colony Treatment Methods to be Utilized in the Response to Stony Coral Tissue Loss Disease (SCTLD)

Prepared By:
Mike Favero & Katie Curtis
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Florida Department of Environmental Protection
Coral Reef Conservation Program
1277 N.E. 79th Street Causeway
Miami, FL 33138

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Table of Contents

Abstract:

The Florida Keys and surrounding bodies of water have become inundated with a deadly coral disease known as Stony Coral Tissue Loss Disease (SCTLD). Recently conducted field studies have shown that a previously designed ointment, known as Base2B, which doses Amoxicillin Trihydrate onto the disease margin of the coral tissue has been effective at halting the disease's progress[1]. In an effort to find other ingredients that might be effective against SCTLD, a variety of products ranging from aquatic natural products, antibacterial essential oils, and weak acids are all being investigated as potential substitutes for Amoxicillin Trihydrate. As each of these 'classes' of treatment have very different physical properties, Ocean Alchemists LLC with resources and equipment donated by CoreRx Pharmaceuticals has worked to develop several ointment formulations capable of delivering each treatment class. All ointments were required to have good adhesion principles underwater and were designed to have targeted release profiles. Dissolution rates were engineered to match that of the effective Base2B ointment which releases an initial burst of treatment then slows over a total of approximately 3days following 1st order dissolution kinetics. The new formulations were also designed to biodegrade into the ocean after total exhaustion of the active compound and to be compatible with whole colony treatment methods. Each class of treatment was studied using dissolution monitoring equipment to determine their respective release rates into the surrounding media. Each ointment studied was capable of producing the desired release profiles while maintaining all of the necessary physical properties required for lesion-based coral treatment. These ointments were then modified and absorbed into both burlap cloths and cotton nets to determine release profiles for larger scale or 'whole colony' preventative treatment efforts. The ointment coated cloths or nets can be anchored over the coral colonies thus saving the SCTLD dive strike teams a significant amount of time underwater performing lesion-based treatments and will theoretically help to reduce the likelihood of disease reinfection at other locations on the colony or reef. This data provides quantifiable proof that the novel ointments created for this study are capable of carrying a variety of possible treatments, both at the lesion level and whole colony treatment scale, that may prove to be effective against SCTLD and potentially other infections that afflict the coral population.

Introduction:

Stony Coral Tissue Loss Disease (SCTLD) has proven to be a devastating disease which has effectively diminished much of Florida's Coral Reef. Once its presence becomes visually apparent, the afflicted colony often parishes within a few days to weeks. If caught early, field research suggests that treatment along the diseased margin with Amoxicillin may be effective at slowing or stopping its progression across individual coral colonies.

This treatment was initially administered successfully in tank bound corals by dosing the water with known concentrations of Amoxicillin. However, in oceanic conditions this becomes an impossibility. To remedy this, the Amoxicillin has successfully been mixed into an ointment known as Base2B which may then be applied to the diseased lesion. Properties of the ointment

allow it to adhere to the coral skeleton adjacent to the diseased lesion. Once in place, the ointment controls the release of Amoxicillin out of the ointment into the surrounding environment. The coral is treated for approximately 3 days before therapeutic dosage concentration is diluted to immeasurable levels in the surrounding water column.

Although effective against SCTLD, Amoxicillin paired with Base2B has several shortcomings. Firstly, Amoxicillin is degraded at a rate of approximately 2% per day[2] in both Base2b and oceanic conditions. The matrix of the Base2B ointment is a foreign material in an oceanic environment and has difficulty degrading. Furthermore, it is a poor carrier for additional treatment options beyond Amoxicillin.

Novel ointments created and outlined within this report sought to improve upon the initial design of Base2B as well as to provide research teams with new ointment delivery systems that will be capable of delivering a wide range of anti-bacterial treatment possibilities at both the lesion level and through whole colony treatment methods.

Part 1 Methods:

Field trials are currently being conducted using multiple variations of the formulations described throughout this report. To ensure that researchers remain blinded until trials conclude, methods will not detail specific concentrations or materials utilized. All novel ointments were developed and compounded in a similar fashion, with the exception of Base2B. This approach was purposeful as the final aim of this collective project is to produce a treatment method and regime capable of scaling to full colony treatment. The final treatment scheme is not yet known; therefore, it becomes paramount that the new delivery systems remain as robust as possible. To meet these demands, a manufacturing approach of utilizing liquification by means of heated stirring was incorporated. This compounding process can be easily scaled up with the use of regularly available pharmaceutical and manufacturing equipment.

Ointments were made by first melting waxy, diluent ingredients followed by the addition of release modifiers and active ingredients. Maintaining the waxy diluents in a liquified state, the mixtures were then blended together by means of overhead stirring. Upon sufficient dispersal, heat was removed while stirring continued. The heated ointments were then packaged into 50mL catheter syringes and left to cool to room temperature. The notion that developed products must be capable of underwater syringe deployment was maintained as a critical performance attribute throughout each formulation's development.

Tested samples were created by extruding approximately 1 gram of specified material from the 50 mL catheter syringe. Samples were then kneaded into the die cavities of dissolution medallions until a smooth, even surface was obtained. Samples of this may be referenced in Image-1 below. Die cavities measured 25 mm in diameter and 2.85 mm in depth.

These dissolution medallions were created so that each sample would have the same surface area exposure for dissolution testing.

Image-1: Dies Filled with ~1 gram of Ointment for Dissolution Testing in the UV-Vis Spectrometer

Dies containing ~1 gram of each sample were then placed into standard USP <711> concave dissolution vessels. Vessels were filled with 1000 mL of deionized (DI) water. Seawater was not utilized during this study due to equipment manufacturer concerns about corrosion over the extended testing period of 3 days. Baths were held at 26.6°C (80°F) for the entirety of each test. Each bath was subject to a simulated current by means of USP <711> apparatus-2 (stainless steel paddles) spinning at 50 rpm, held approximately 2.5 cm above the ointment filled dies. A single Pion UV-Vis Spectrometer probe was present in each testing bath. Probes were set to collect a data point every 10 minutes for the first 4 hours followed by every 30 minutes for the following 68 hours. Measurement wavelengths, standard curves for comparison, and probe tip pathlengths were unique to each study. Please reference subset method specifics for detailed information regarding such. Each ointment studied was conducted in either N=3 or N=6 samples. Samples were then averaged to produce the various graphs located throughout this report.

Image-2: 1000 mL UV-Vis Dissolution vessel with Die Cavity Containing ~ 1 gram of Ointment Sample

Part 1 Results:

Each data set provides results in terms of '%Released'. This number is calculated by determining the amount of active compound present in each sample divided by the amount of dissolution media (1000 mL). If all of the possible active compound present were to be released into the dissolution media within each sample, then the %Released would equal 100% dissolution. Please note that emphasis should be placed on the release profile rather than reaching 100% dissolution due to the knowledge that each ointment is capable of carrying varying quantities of active compound. The %dissolution curve with respect to time is the true value of these studies as this rate of release is heavily dependent on dissolution modifying excipient concentrations rather than active compound concentration.

Due to the multitude of ointment designs and associated testing parameters, the results have been sequestered into individual subsets. Each subset outlines the specific methods and results associated with each class of active ingredient: A- Amoxicillin, B- Aquatic Natural Products, C- Essential Oils, & D-Acidic.

Part 1 Subset A: Amoxicillin in Novel Biodegradable Ointment Compared Against Base2B and Neat Shea Butter

Part 1 Subset A Methods:

Each sample tested was aliquoted from a larger batch size in order to provide an accurate representation of the ointment. Amoxicillin in Base2B was compounded in its traditional fashion where the placebo ointment was first created, followed by the addition of Amoxicillin hand mixed into the ointment in a fashion congruent to what is done during field studies. For the remainder of the samples (amoxicillin in the novel biodegradable ointment, neat shea butter analyzed by UV-Vis, and neat shea butter analyzed by HPLC), the ointments were heated to the point of liquification. This temperature ranges from 35-55ºC depending on ingredient concentrations. Amoxicillin was then added and mixed by magnetic stir bar. Heat was removed while stirring continued.

The ointments were then left to 'set' for a minimum of 24 hours to ensure that all excess energy from heating was dissipated and the final ointment conformation had been reached. Samples removed for testing were aliquoted at room temperature.

UV measurements performed by the Pion UV-Vis spectrometer utilized the 230 nm wavelength paired with 5 mm pathlength tips fitted to each probe to quantify Amoxicillin present in solution. UV measurements performed by HPLC analysis were taken on individual samples aliquoted from the bulk dissolution vessel (1000 mL) at the time points shown in Graph-1. These samples were pulled in 4 mL aliquots and immediately placed into 2-5ºC storage to slow degradation. All samples were pulled from 2-5ºC storage at the 72 hour point, filtered through a 0.2 micron filter and vialed for HPLC analysis.

Amoxicillin in Neat Shea Butter Utilizing HPLC Study Methods & Parameters:

A small stir bar was added to the vessel and left to stir at a slow laminar flow throughout testing. The sample was stored in the absence of UV light at room temperature. HPLC samples were removed at various time points and prepared by filtering approximately 10 mL of sample through a 0.2 micron Nylon filter and filled into appropriately labeled HPLC vials for testing.

Parameter	Value
HPLC #	72
Flow Rate	1.5 mL/minute
Injection Volume	100 µL
Runtime	26 min
Wavelength Monitored	260 nm
Column Temperature	40°C
Pump Mode	Gradient
HPLC Column	Agilent Eclipse Plus C18 5 um x 4.6 mm x 150 mm SN:USUXB12969

Table 1: HPLC Testing Specifications Regarding Amoxicillin in Neat Shea Butter Analyzed by HPLC

Sample Run Time (minutes)	Mobile Phase A (2.72 g/L Monobasic Potassium Phosphate Adjusted to pH 5)	Mobile Phase B (Methanol)
0	97%	3%
10	97%	3%
22	75%	25%
26	97%	3%

Table 2: HPLC Sample Run Mobile Phase A and Mobile Phase B Gradient Concentrations with Respect to Sample Run Time

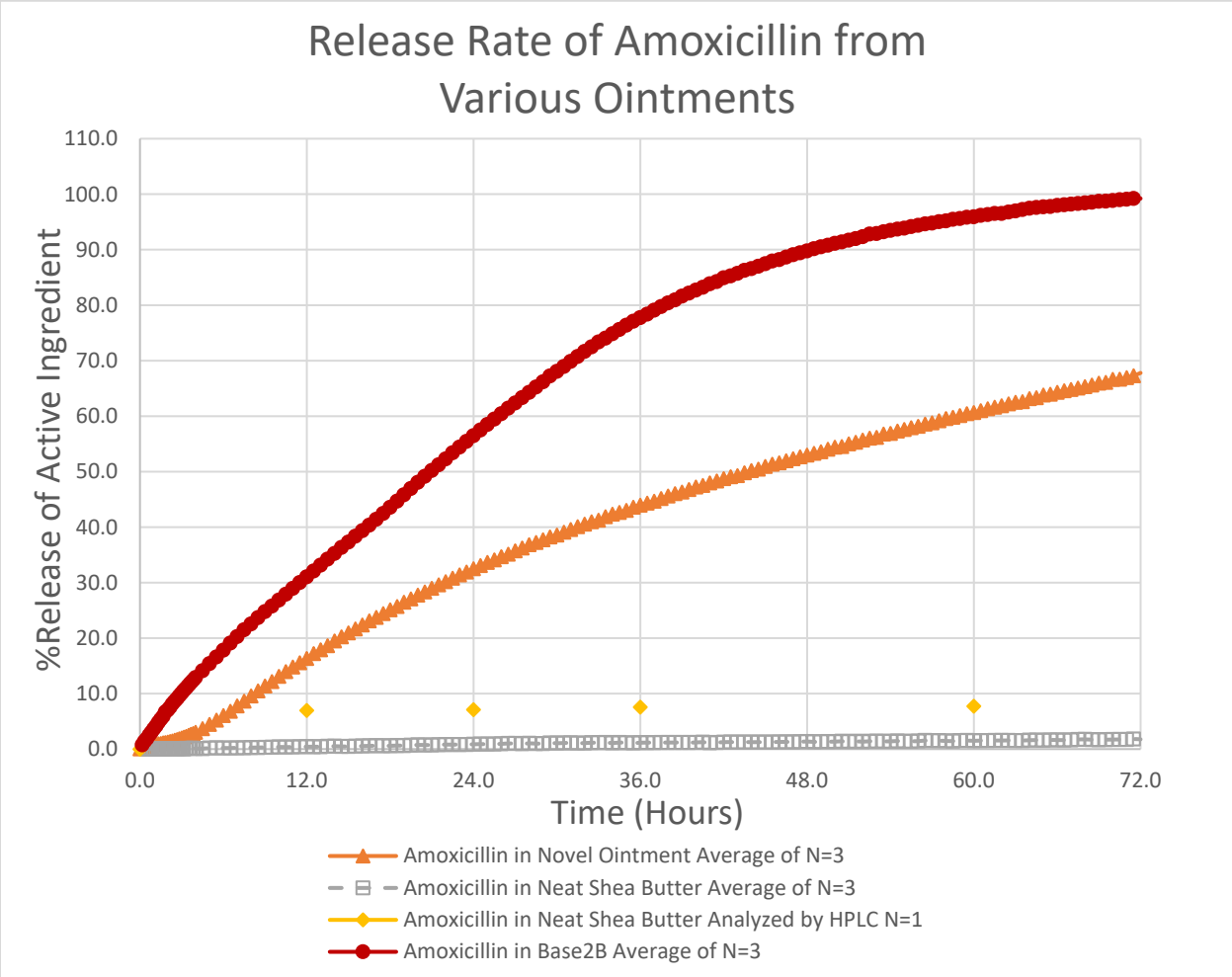
Part 1 Subset A Results:

The data presented in Graph-1 below represents the results gathered from release test studies for Amoxicillin Trihydrate from the new biodegradable ointment matrix. The primary objective was to create a novel ointment which could both carry and deliver Amoxicillin Trihydrate in a similar fashion to the Base2B formulation currently utilized in field studies. Although possessing a slower release, the novel ointment possesses the 1st order active compound release curve found with Base2B. Currently, it is unknown if a longer sustained release or a short, concentrated burst of Amoxicillin is more effective. Field trials which utilize Amoxicillin in the novel biodegradable ointment may provide new evidence to support either of these possibilities.

A regression analysis of r^2>0.995 provides evidence that the methods were capable of quantifying dissolved Amoxicillin from 0% to 150% of the expected value. It should be noted that the primary objective of these studies was to monitor the release rates rather than to quantify the total amount of Amoxicillin released. Sample concentrations were calculated to be approximately 12.9% Amoxicillin, slightly above the commonly targeted field application

concentration of 11%. Samples were weighed and compared to the known standard curves of Amoxicillin in DI water. Samples were then combined and averaged to produce the results.

Additionally, Amoxicillin Trihydrate in neat shea butter was monitored by two separate means. Shown below are the results of both the active UV-Vis data utilized to report both the Base2B results and the novel biodegradable ointment results. The results of a previous study conducted utilizing HPLC analysis have also been included. In the latter, water samples were taken at the time points shown below and analyzed for Amoxicillin concentration using an HPLC.



Graph-1: Release Rate of Amoxicillin from Various Ointments at 80ºF over 72 hours

Interpretation of either testing method supports the same conclusion which suggests that neat shea butter is incapable of releasing Amoxicillin beyond particles bound to its surface, up to only 7% of the total Amoxicillin concentration. This is due to the fact that neat shea butter is an oil-based product which is hydrophobic and therefore unable to release the amoxicillin mixed within the matrix into an aqueous environment without the addition of other excipients. If positive field results are reaped utilizing such, it may suggest a method of action which differs from that found in Base2B or the novel biodegradable ointment.

Part 1 Subset B: Aquatic Based Natural Product in Novel Biodegradable Ointment Compared Against Base2B

Part 1 Subset B Methods:

To monitor the release rate of any potential water-soluble extract, a blue dye (Blue Dye#1) was impregnated into the novel ointment in place of an extract. Blue Dye#1 was chosen as it is both readily water soluble, and easily measurable using UV spectroscopy. Known quantities of the dye were added to the ointment and quantitated against a standard curve which spanned the expected release rate of the dye from 0%-125% with a correlation of determination (r^2) > 0.995. The results were monitored by UV spectroscopy utilizing a Pion UV-Vis Spectrometer paired with dissolution probes attached to fiber optic cables to conduct real-time measurements of individual dissolution vessels.

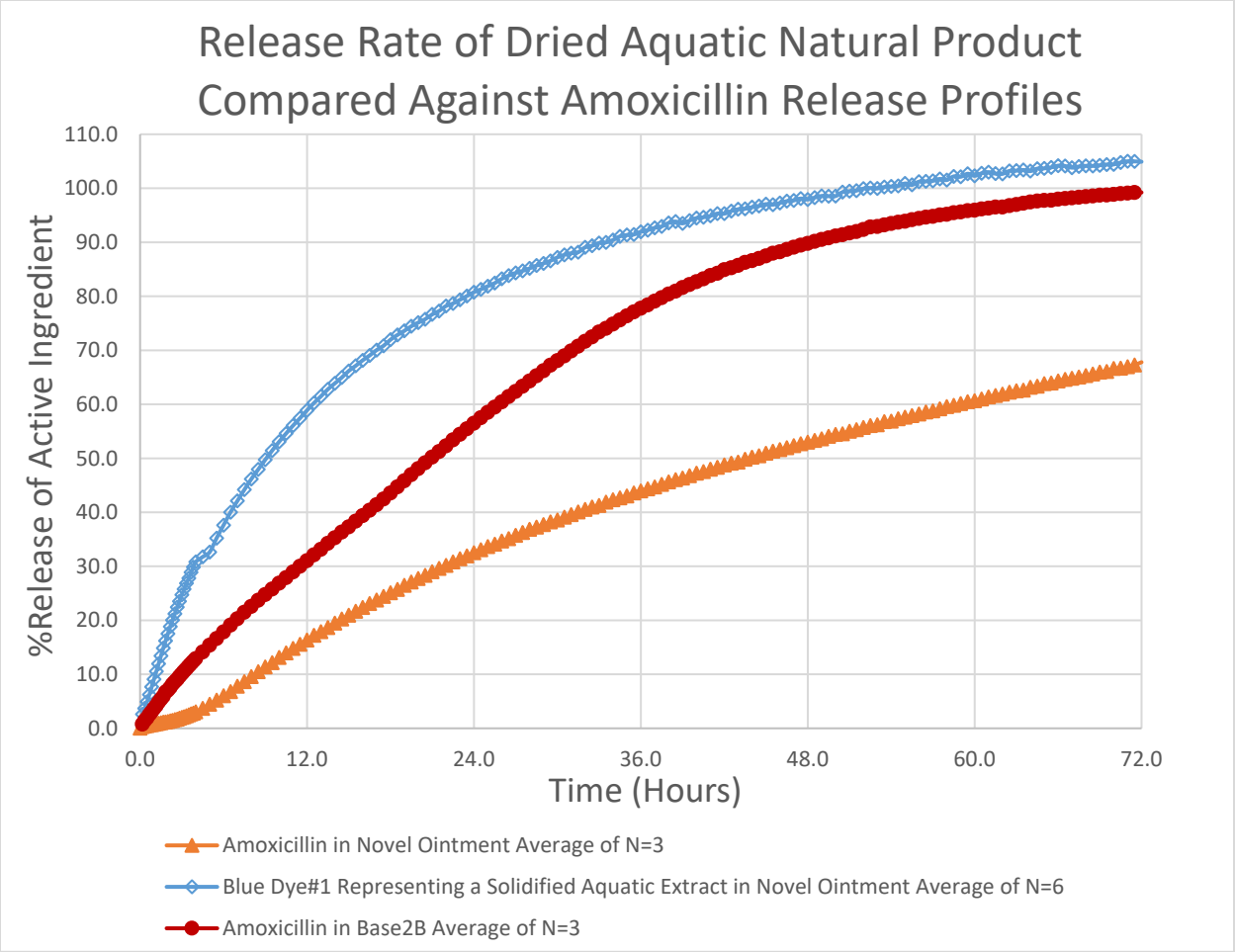
The Blue Dye#1 was measured at 630 nm wavelength paired with 10 mm pathlength tips fitted to each probe. It should be noted that the Blue Dye#1 was present in the ointment at a 1% weight/weight concentration. This would likely be near the low end of aquatic extract loaded into the placebo ointment. Thus, the model is designed to be utilized as a release profile guide for field scientist to determine application and monitoring schedules.

Part 1 Subset B Results:

In the ongoing attempt to find compounds capable of effectively treating SCTLD, Ocean Alchemists with resources, knowledge and equipment donated by CoreRx Pharmaceuticals created a novel biodegradable ointment designed to carry and release dry natural product extracts. The goal of this process was to create a robust ointment capable of carrying a variety of water-soluble extracts. This is in response to the multitude of crude aquatic extracts which possess antibacterial or antiseptic principles.

The addition of dry aquatic natural products significantly increased the solid concentration in the ointment and thus modifications were needed to maintain the ointment's physical properties. Both thermal plasticity and ability to extrude from a syringe were critical performance parameters for ointments in lesion-based treatment methods.

The targeted profile for the ointment was set to follow the positive field test results gathered from the combination of Base2B and Amoxicillin which both demonstrate a 1st order release profile. Graph-2 located below provides the results of the study.



Graph-2: Release Rate of Blue Dye#1 Substituted for a Dried Aquatic Natural Product from Novel Ointment at 80ºF over 72 hours Compared Against Various Amoxicillin Ointment Release Profiles

The release rate for dry natural products is slightly faster than that of the Amoxicillin, reaching 100% release in 2 days rather than the targeted 3 days for Amoxicillin, however the desirable 1st order release profile was achieved. If any water-soluble natural product extracts prove to have efficacy against SCTLD, this release profile can be later adjusted to optimize the dosing rate for that specific active ingredient.

Part 1 Subset C: Essential Oils in Novel Biodegradable Ointment Compared Against Base2B

Part 1 Subset C Methods:

Known quantities of the essential oil extract were added to the novel ointment and quantitated against a standard curve which spanned the expected release rate of the oil from 0%-125% with a correlation of determination (r^2) > 0.995. The results were monitored by UV spectroscopy utilizing a Pion UV-Vis Spectrometer paired with dissolution probes attached to fiber optic cables to conduct real-time measurements of individual dissolution vessels. The essential oil was measured at 220 nm wavelength paired with 2 mm pathlength tips fitted to each probe. The oil was present in the ointment at a 18% weight/weight concentration.

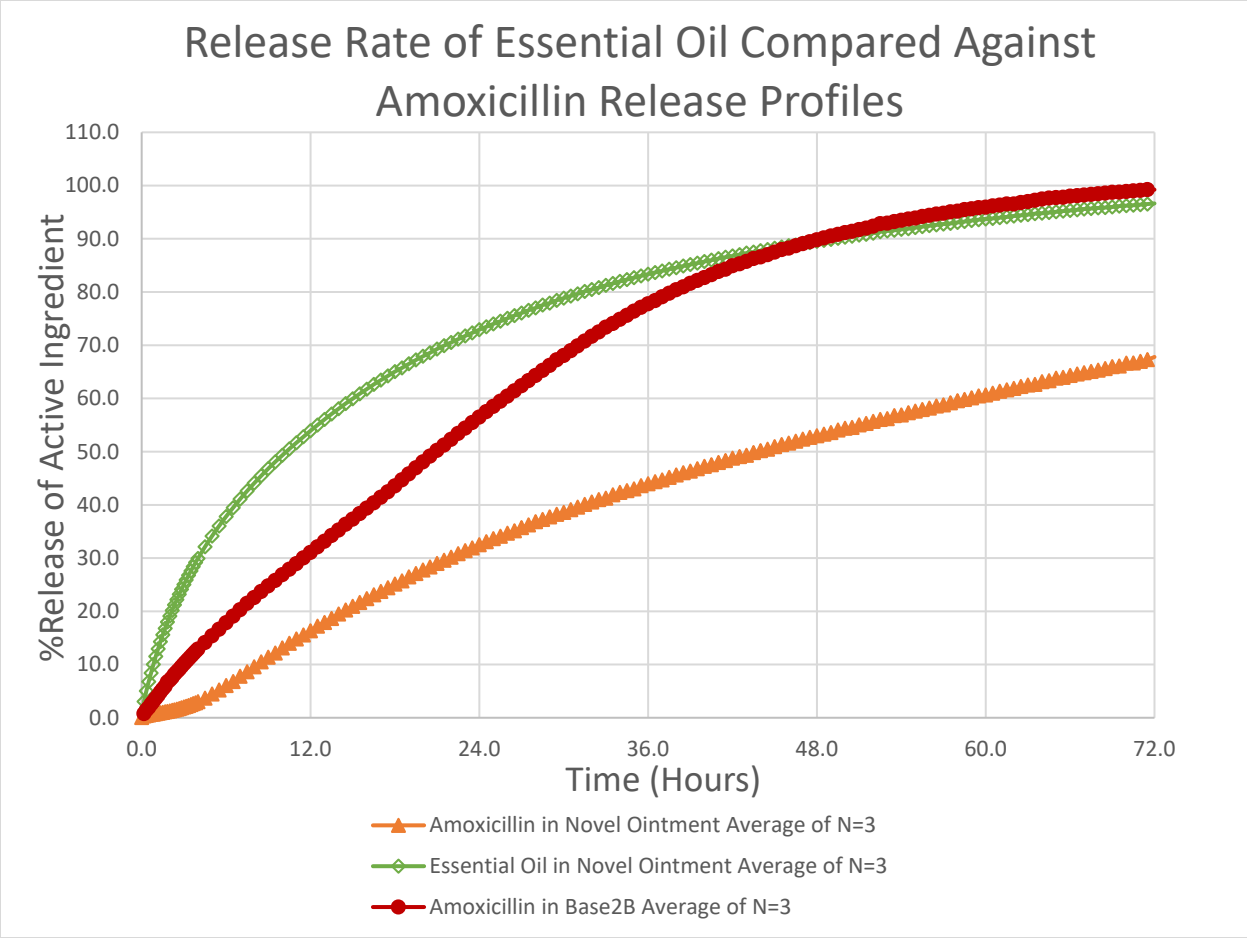
Part 1 Subset C Results:

In an effort to research new compounds that may be utilized for the treatment of SCTLD, combinations of a variety of essential oils may prove to be effective when applied to afflicted coral tissue as many of them possess significant antibacterial properties. Due to the oleophilic nature of the essential oils however, multiple modifications to the biodegradable ointment were necessary.

Ointment adhesion was a paramount factor for each formulation, therefore many of the design modifications for carrying oil based active ingredients were centered around maintaining good adhesion while adding a significant liquid concentration.

Additionally, adding the essential oils initially weakened the ointment's thermal plasticity. This was compensated for with the addition of another base material that possessed a higher transition temperature. Extrudability of the product from a syringe as well as the transition temperature of the ointment both became critical factors during the design process. This also led to large adjustments in the ointment's release modifying excipients in order to maintain the desired release profile.

The final design of the ointment had all of the necessary properties; good adhesion, extrudability from a syringe, the desired plasticity at the specified treatment temperatures (18-30°C) and a dissolution profile rate that mimicked the targeted release profile.



Graph-3: Release Rate of an Essential Oil from Novel Ointment at 80°F over 72 hours Compared Against Various Amoxicillin Ointment Release Profiles

Graph-3 gives quantifiable evidence that the release modifiers make it possible for the oleophilic active ingredients to migrate through the ointment and release into the surrounding water column. Again, the ~3 day 1st order release profile was targeted. This treatment regime is designed to deliver an initial burst of concentrated active which gradually weakens over a 3 day period.

Part 1 Subset D: Acidified Biodegradable Ointment Compared Against Base2B

Part 1 Subset D Methods:

Known quantities of the blue dye were added to the ointment and quantitated against a standard curve which spanned the expected release rate of the dye from 0%-125% with a correlation of determination (r^2) > 0.995. The results were monitored by UV spectroscopy utilizing a Pion UV-Vis Spectrometer paired with dissolution probes attached to fiber optic cables to conduct real-time measurements of individual dissolution vessels.

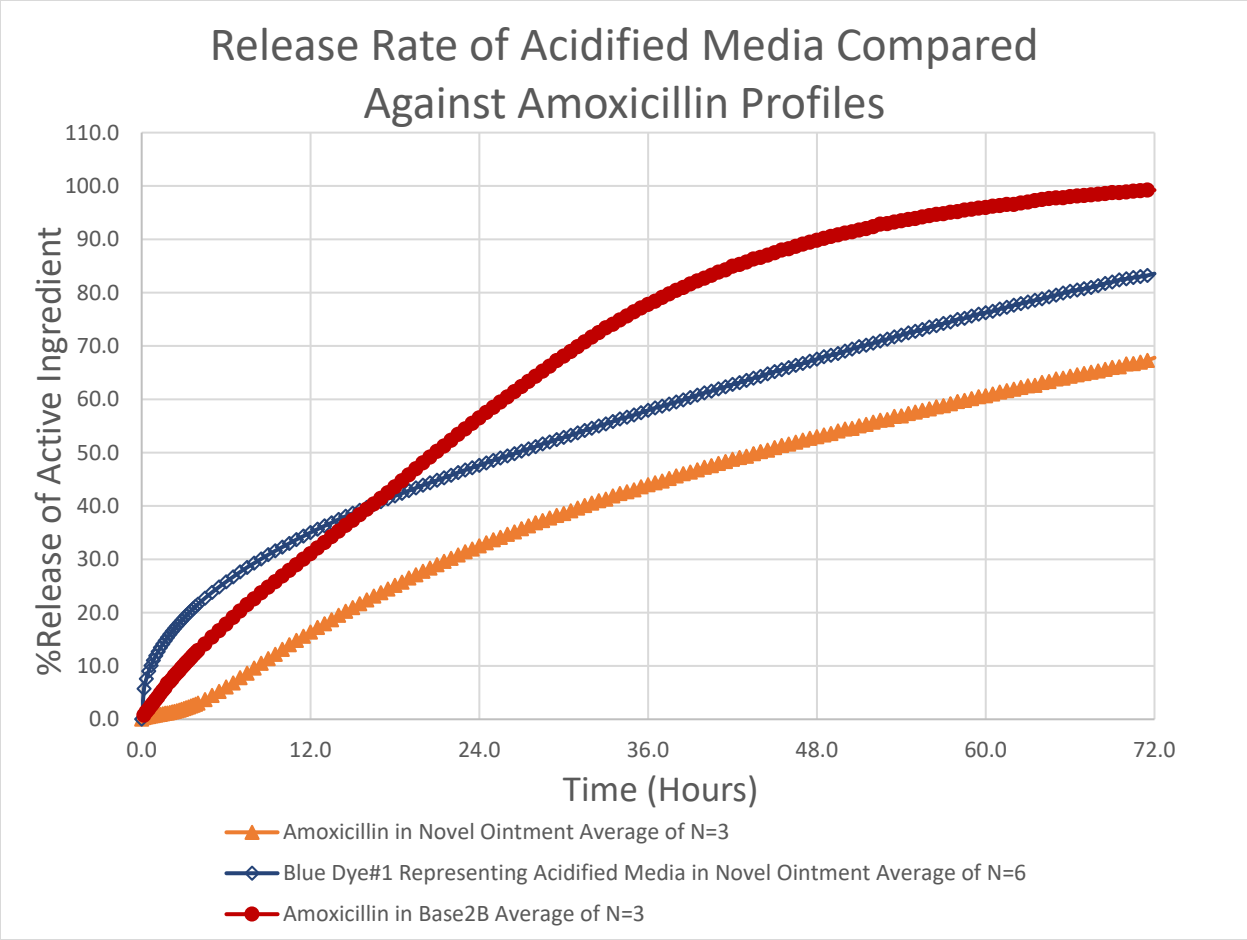
Monitoring the release of the acidified ointment became challenging due to UV probe limitations. To ensure comparability amongst samples, each study utilized a 1 gram sample paired with the die apparatus which standardized the sample surface area. Within these guidelines, the acid did not exhibit a strong enough absorbance signal in a range that could be accurately quantified using Pion UV-Vis spectrometer equipment. It was determined the amount of acidified ointment needed to produce a signal within a quantifiable range was beyond rationale.

A unique solution to the conundrum again utilized Blue Dye#1. Measurements were taken at 630 nm wavelength paired with 10 mm pathlength tips fitted to each probe. Ointment used for testing was compounded by first dissolving the blue dye into the acid. The dyed acid was then absorbed into carrying microspheres followed by dispersal into the ointment. Due to their mutual oleophobic nature, neither is capable of dispersing into the oil phase in quantifiable concentrations. As both are freely soluble in aqueous environments, monitoring the release rate of the blue dye provides a reasonable estimation of the acid's release rate.

Part 1 Subset D Results:

Recent discussions have suggested that a brief acidification period in the microenvironment along the lesion border may be an effective treatment against SCTLD. Unfortunately, this is a difficult study to determine in an in-vitro tank environment as the goal is not to acidify the entire water column. To test this theory a novel ointment capable of maintaining a localized acidic condition at its point of contact was needed. Through multiple iterations of trial and error a successful ointment was created. The key was to sequester the acidic solution from the oleophilic delivery system to minimize resulting negative interactions between the acid and the ointment base. This was accomplished by impregnating a secondary substrate with the acidic solution and blue dye followed by the addition of the impregnated microspheres into the ointment. This created a protective barrier for the acid to escape the oleophilic ointment, effectively minimizing interaction with the ointment matrix before deployment. Once adhered, the release modifying excipients within the ointment become hydrated which provide a pathway for the acid and blue dye to escape the microspheres embedded within the matrix. The resulting ointment is believed to be capable of syringe extrusion with superior adhesion in comparison to Base2B.

The release rate of acidified media shown in Graph-4 perfectly mimics the successful release of Amoxicillin from both Base2B and the new biodegradable ointment. This demonstrates that the use of microspheres can be utilized to provide protection of an acidified active ingredient until deployment and that this new biodegradable ointment can be utilized for this class of active ingredients against SCTLD.



Graph-4: Release Rate of Blue Dye#1 Solubilized into Acidified Media from Novel Ointment at 80°F over 72 hours Compared Against Various Amoxicillin Ointment Release Profiles

Part 2 Methods:

Methods of creation, application and testing were constant throughout Part 2 Subsets A, B & C. Successful ointments for each of the active compound classes outlined in Part 1 were modified to whole colony treatment delivery methods. Trials included the continued modification of multiple ointments loaded with the current lesion level successful active ingredient, Amoxicillin Trihydrate, as well as both water-soluble and oil-soluble active compounds. Unfortunately, early failure of the acidified ointment delivery system in the field indicated that continued research into full colony treatment utilizing such is not advisable for further investigation at this time.

Two separate types of materials were coated for each ointment system studied. Burlap and cotton were chosen due to their tough physical properties paired with their biodegradation capabilities in the undesirable event that the treatment apparatus is lost. The first material is a tightly woven burlap cloth, the second is a cotton net with box shaped holes approximately 2 centimeters in length. Both are designed to be anchored onto a coral colony, covering the colony in a blanket like fashion. Both are also designed for short treatment regimens ranging no more than a couple of days in order to minimize impacts to the coral reefs.

Both burlap cloth and cotton nets were soaked into the ointments after their final composition was fully compounded. The ointments were left in a liquified state during the process to provide improved absorption into the fibers. Upon removal of the materials from the liquified ointments, both the cloths and nets were held in a hang dry fashion, allowing excess ointment to be removed by a dripping process while the ointment solidified. Set sizes of cloths and nets with known weights were utilized to ensure the weight fraction of the ointment was known. Weight of applied ointment ranged from 0.5 grams - 1.5 grams per sample.

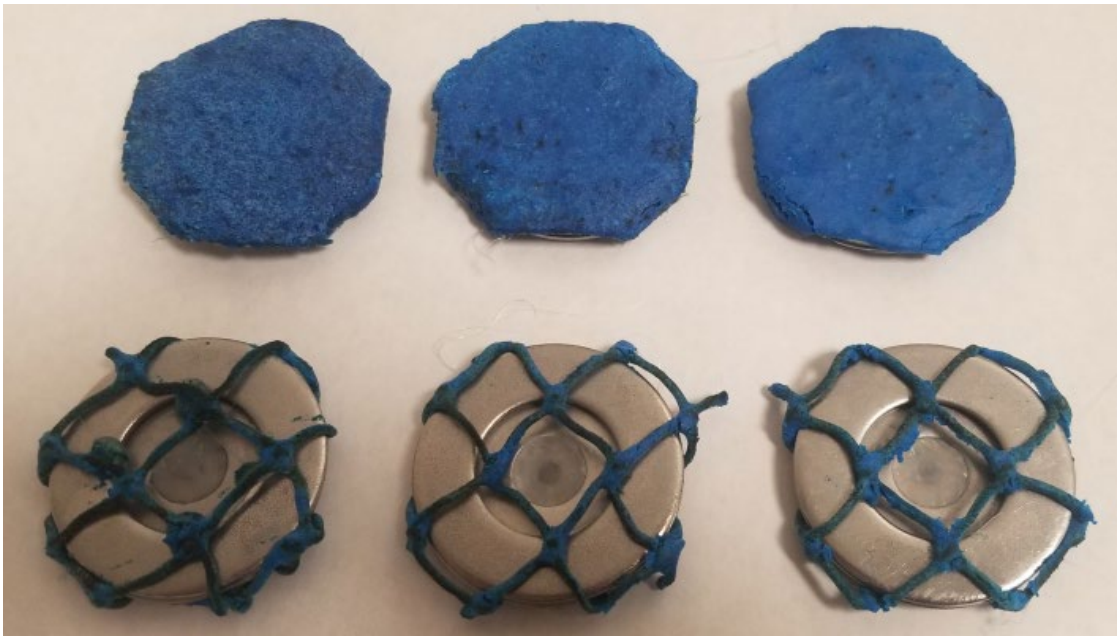


Image-3: Ointment coated burlap cloths and cotton nets on 50.8 mm stainless steel medallions for dissolution testing

Both cloths and nets with applied ointment were mounted to stainless steel medallions. Medallions were added to pharmaceutical USP<711> dissolution vessels filled with 1000 ml of DI water. Sea water /saltwater was not utilized due to equipment manufacturer concerns over leaving UV probes in simulated sea water over extended time periods. Water currents were simulated through the use of USP<711> apparatus#2 (stainless steel paddles) set to 50 rpms held at approximately 2.5 cm above the medallions throughout all testing. Water temperature was held at 26.6ºC (80ºF) for the entirety of each study. A single Pion UV-Vis Spectrometer probe was present in each testing vessel. Probes were set to collect a data point every 10 minutes for the first 4 hours followed by every 30 minutes for the following 44 hours. Measurement wavelengths, standard curves for comparison, and probe tip pathlengths were unique to each study. Please reference sub-study method specifics for detailed information regarding such. Each ointment studied was conducted in either N=3 or N=6 samples. Samples were then averaged to produce the various graphs located throughout this report.

Part 2 Results:

The data presented below demonstrates that the formulations on both burlap cloths and cotton nets can be utilized to modulate the release profile for a variety of active ingredients anywhere from 12 hours to ~ 3 days to best suit the optimized dosing rate for each active ingredient in a whole colony treatment regime. The release profiles shown are what will determine when a treatment system should be removed, along with aiding to determine the ideal dosage rate for potential active ingredients. The data presented in this section can be used to optimize the time these systems are applied to the reef beyond exhausting their medication; doing so will help to reduce any unnecessary impact on the coral colonies and surrounding organisms.

Each data set provides results in terms of '%Released'. This number is calculated by determining the amount of active compound present in each sample divided by the amount of dissolution media (1000 mL). Please note that emphasis should be placed on the release profile rather than reaching 100% dissolution due to the knowledge that each ointment is capable of carrying varying quantities of active compound. The %dissolution curve with respect to time is the true value of these studies as this rate of release is heavily dependent on dissolution modifying excipient concentrations rather than active compound concentration. The release rates for each class of active ingredient are presented in the subsets below: A- Amoxicillin, B- Water Soluble Active Compounds & C- Oil Soluble Active Compounds.

Many of the parameters for lesion-based ointments were not required when designing formulations for whole colony treatment regimens such as adhesion to a coral substrate, and extrudability. Instead paramount importance was placed upon optimizing drug release rates for each ingredient out of both burlap cloths and cotton nets. Multiple ointment formulations were created for Amoxicillin to demonstrate that adjustments can be made to the excipients in order to modulate the release profiles as needed. Field studies will be required to determine how long the cloths or nets can realistically be deployed as well as the most effective dosing rate for each active ingredient; the formulation can then be optimized accordingly.

It should be noted that while ointments modified for both burlap cloths and cotton nets were able to be formulated with the desired release profiles, nets appear to be a more effective method for whole colony treatment. The cotton nets were more readily able to imbibe the ointment into the fibers of the material which allowed for a more steady release rate of the active compounds. Nets also present less potential risk to the coral colonies than the burlap cloths as they are less likely to inhibit the coral's ability to filter feed. Cotton nets are also far less likely than burlap cloth to reduce the amount of light available for zooxanthellae photosynthesis while actively deployed.

Both methods of whole colony treatment studied in this report present promising opportunities for large scale treatment against SCTLD and should be further investigated for efficacy in the field. Both of these methods have the potential to save a significant amount of time underwater administering treatment and may drastically reduce the likelihood of reinfection of the disease throughout the coral reef by providing a preventative treatment against SCTLD.

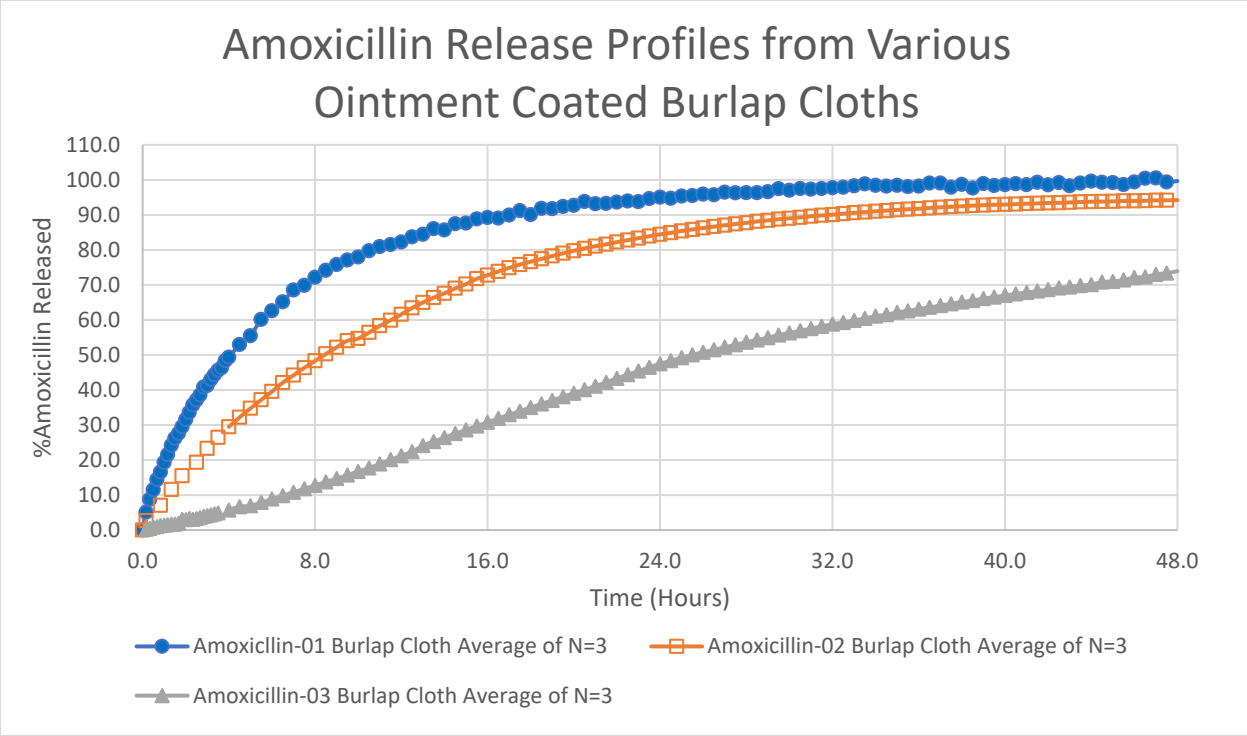
Part 2 Subset A: Amoxicillin from Burlap Cloths and Cotton Nets

Part 2 Subset A Methods:

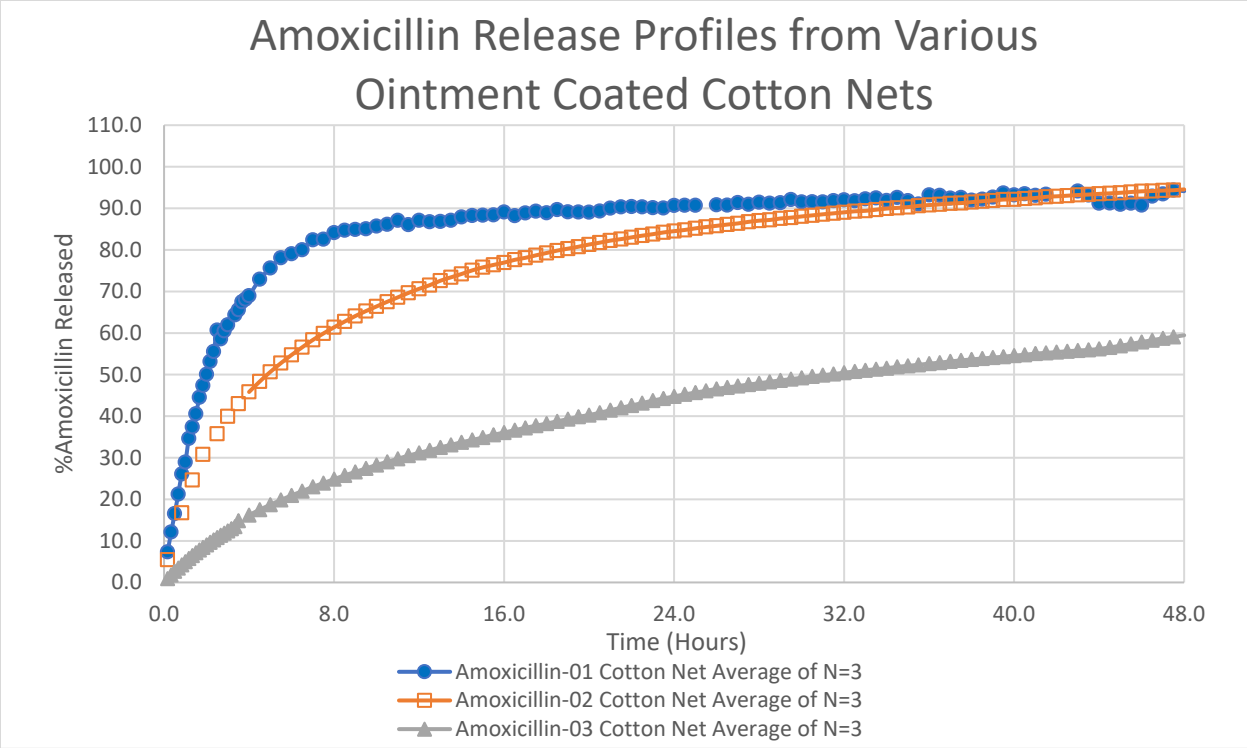
Known quantities of Amoxicillin were added to the ointment and quantitated against a standard curve which spanned the expected release rate of the Amoxicillin from 0%-150% with a correlation of determination (r^2) > 0.995. The results were monitored by UV spectroscopy utilizing a Pion UV-Vis Spectrometer paired with dissolution probes attached to fiber optic cables to conduct real-time measurements of individual dissolution vessels. The Amoxicillin was measured at 230 nm wavelength paired with 5 mm pathlength tips fitted to each probe. The Amoxicillin was present in the ointment at an 11% weight/weight concentration.

Part 2 Subset A Results:

A three-day active compound release profile appears to work best for lesion level application, however it is unknown if a different profile will be required for large scale whole colony treatment methods. Multiple ointments designed for dispersion from cotton nets and burlap cloths were created for the targeted release of ~ 11% weight Amoxicillin Trihydrate in order to demonstrate the range of release rates that can be achieved by altering the concentration of certain excipients. The results in Graph-5 show that a range from ~12 hours to ~3 days is possible with these formulation adjustments.



Graph-5: Release Rate of Amoxicillin out of Ointment Coated Burlap Cloths at 80°F over 48 hours



Graph-6: Release Rate of Amoxicillin out of Ointment Coated Cotton Nets at 80ºF over 48 hours

The results presented in graphs 5 & 6 provide quantifiable proof that either burlap cloth or cotton nets are viable options for continued study with Amoxicillin as they both display 1st order release kinetics. However, ointment coating of the burlap cloths proved challenging as penetration into the burlap fibers was less effective in comparison to the cotton nets. Several 'dips' into heated ointment were necessary to properly coat the burlap cloths where the cotton nets were easily and ready coated upon a single dip.

Part 2 Subset B: Water-Soluble Active Compound from Burlap Cloths and Cotton Nets

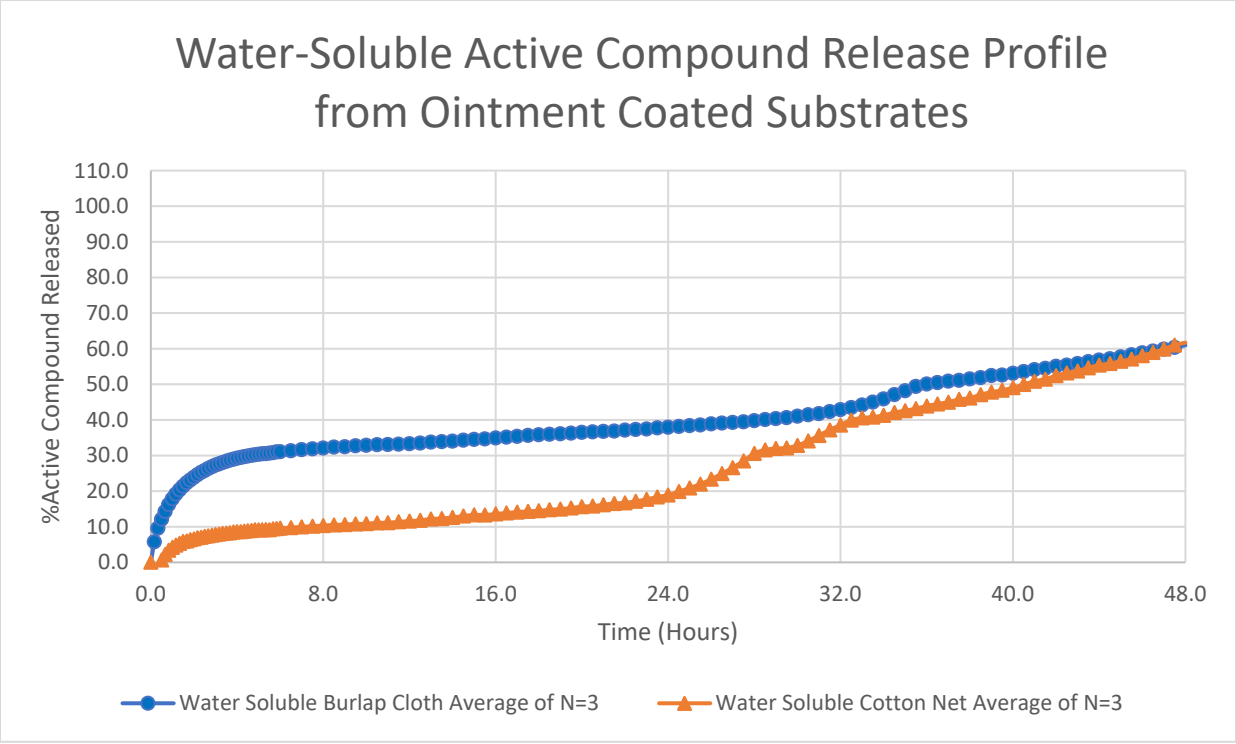
Part 2 Subset B Methods:

Known quantities of the water-soluble active compound were added to the ointment and quantitated against a standard curve which spanned the expected release rate of the compound from 0%-125% with a correlation of determination (r^2) > 0.995. The results were monitored by UV spectroscopy utilizing a Pion UV-Vis Spectrometer paired with dissolution probes attached to fiber optic cables to conduct real-time measurements of individual dissolution vessels. The water-soluble compound was measured at 245 nm wavelength paired with 2 mm pathlength tips fitted to each probe. The water-soluble compound was present in the ointment at a 25% weight/weight concentration.

Part 2 Subset B Results:

Provided below are the 25% weight water-soluble active compound release results from both burlap cloths and cotton nets. This provides further proof of the cotton net's superior ability to absorb the ointment more effectively in comparison to the burlap cloths. The slower release from nets utilizing the identical ointment formulation is indicative of deeper penetration which provides a thicker, more torturous path for active compound diffusion thus creating a more desirable dosing rate.

Challenges related to coating the cloths with ointment in Part 2 Subset A were again present in Part 2 Subset B. During dissolution studies, a sizable quantity of small fibrous particulate regularly pulled away from the burlap cloth. This did not have a significant effect within these studies as each vessel was set to a finite volume, so any active compound released from detached ointment was still quantified. However, in field studies any material which becomes unanchored to the treatment apparatus would no longer be effectively treating. Therefore, cotton nets again proved to be a more effective treatment method than burlap cloths for whole colony treatment investigations due to their ability to maintain ointment within the fibers of the material.



Graph-7: Release Rate of a Water-Soluble Active Ingredient out of Coated Burlap Cloths and Cotton Nets at 80°F over 48 hours

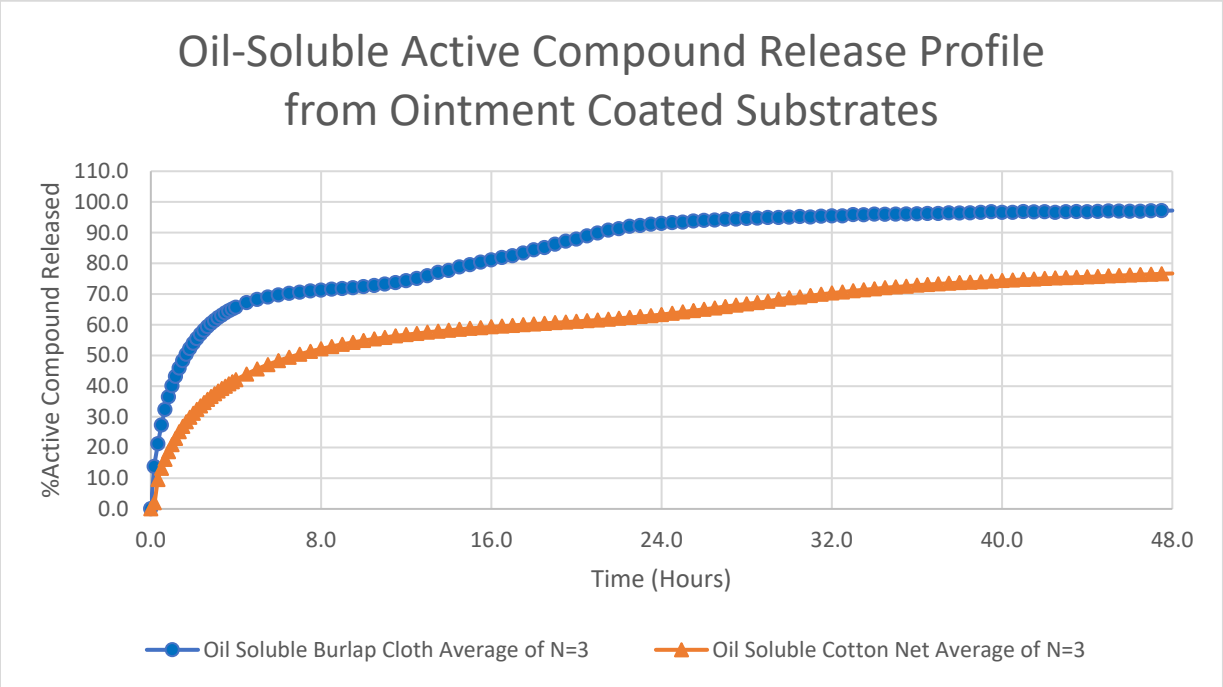
Part 2 Subset C: Oil Soluble Active Compound from Burlap Cloth and Cotton Nets

Part 2 Subset C Methods:

Known quantities of essential oil extract were added to the ointment and quantitated against a standard curve which spanned the expected release rate of the oil from 0%-125% with a correlation of determination (r^2) > 0.995. The results were monitored by UV spectroscopy utilizing a Pion UV-Vis Spectrometer paired with dissolution probes attached to fiber optic cables to conduct real-time measurements of individual dissolution vessels. The essential oil was measured at 270 nm wavelength paired with 2 mm pathlength tips fitted to each probe. The oil was present in the ointment at an 18% weight/weight concentration.

Part 2 Subset C Results:

An ointment capable of carrying oil-soluble 18% weight active compounds was created, applied to both burlap cloth and cotton nets followed by UV-Vis Spectrometer monitoring.



Graph-8: Release Rate of Oil Active Ingredient out of Coated Burlap Cloths and Cotton Nets at 80°F over 48 hours

Results indicate that ointment coated cloths and nets provide an initial burst and are followed by a 1st order release extending beyond the 48 hours monitored. Continuing the trend seen in the water-soluble results, ointments absorbed into burlap cloth provided quicker release results compared to ointments absorbed into cotton nets. Mentioned in Part 2 Subset B, this is likely indicative of enhanced ointment penetration into the cotton net fibers in comparison to the burlap cloth creating a more torturous path of diffusion and thus a more desirable release profile.

Discussion:

It should be noted that oceanic conditions rarely provide stable currents and will likely vary in strength from those utilized for testing. The various release studies documented in this report are intended to serve as a guide rather than absolute. These methods may be utilized to help field scientists determine more accurate treatment regimens. Most importantly, these results provide quantifiable proof that each of the ointments created in this study are capable of delivering steady reproducible treatments over 12 hours – 3 days which can be adjusted to accommodate the optimal dosing rate of any potential active ingredients.

Furthermore, each novel ointment created by Ocean Alchemists with knowledge and resources donated by CoreRx Pharmaceuticals may be designed to carry a multitude of active ingredients. The ointments studied span across oleophilic, dry hydrophilic, liquid hydrophilic, and acidified media. Therefore, any potential antibacterial, antiseptic, or antibiotic ingredients that falls within one of these classes may be quickly compounded and deployed with reasonable confidence of its release profile. Additionally, if moderate success with any of these ointments is documented to be effective against SCTLD, these profiles provide a zero point from which the effective treatment may be manipulated to either increase or decrease their respective release profiles.

A continued issue which sometimes propagates in the field is reinfection of the disease at locations on the reef other than the original treated margins. The percentage of reinfection is believed to be dependent on a multitude of factors including the coral colony density, currents and a range of other variables. The utilization of whole colony treatment methods like the burlap cloths and cotton nets investigated in this study will work to time release the most effective active ingredient over large areas including those currently unaffected by the disease thus providing a preventative treatment method. The ointment coated cloths or nets can be anchored over the coral colonies which should save the SCTLD dive strike teams a significant amount of time underwater performing lesion-based treatments and will theoretically help to reduce the likelihood of disease reinfection at other locations on the colony or reef.

It should be noted that nets consistently proved to have better absorption of the material and thus created more desirable release rates. It is understood that utilizing either cloths or nets as a means of full colony treatment may also present some adverse effects. However no other large-scale treatment method for treating coral reefs exists at this time. Limiting the time period treatment apparatuses are anchored, along with complete biodegradation of the materials should help to mitigate most risks. Overall, we believe that the combination of the ointments studied in this research paired with the right active ingredient can be utilized in both lesion based and whole colony treatment methods to have a large-scale positive impact for all corals affected by SCTLD.

References:

1. Neely, K. (2018). In Situ Disease Intervention (June 2018)
2. Favero, M., Balut, K., & Levine, M. (2019). Amoxicillin Trihydrate Stability in Correlation with Coral Ointment Batch#18006-B and Simulated Seawater.