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Release Kinetics of Methylene Blue in Pluronic F-127 Hydrogel

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Abstract

"Between January 1998 and March 2000, veterinarians at the ASPCA National Animal Poison Control Center (NAPCC) consulted on more than 1,050 cases of accidental exposures to acetaminophen and 1,100 cases of ibuprofen ingestion in dogs and cats" (Richardson, 2000).

NSAID toxicity has become an increasingly common occurrence in small companion animals within the last decade due to widespread usage (Zhou, Boudreau, & Freedman, 2014). Depending on the specific drug, treatment is centered around early decontamination and prevention of kidney and hepatic damage, and methemoglobanemia. Currently, methemoglobanemia is treated with intravenous delivery of methylene blue and less commonly through oral administration. While these methods have been shown to be successful, congenital forms of methemoglobinemia are more difficult to manage as reoccurring treatments are imperative.

A biocompatible hydrogel composed of Pluronic F127 and methylene blue was created at various percent weight ratios to analyze release kinetics and determine the most effective composition for clinical treatment of methemoglobinemia. Using a hydrogel for localized delivery of methylene blue allows for long-term release of the drug, making treatment more feasible for affected patients and their owners.

Initially, samples were created using 15-20 percent weight Pluronic F127 and deionized water in 20 mL vials. The polymer was allowed to dissolve by mechanical stirring using a magnetic stir bar for 24 hours. Once completely dissolved, 7.8%, 15.6%, or 31.2% methylene blue was added to each vial. Samples were placed in an oven at 37 degrees Celsius. Samples

were observed on 60-second intervals to check for gel formation. At this critical point, the solution was no longer viscous. Clinically favorable samples achieved a gel formation time at less than 10 minutes while other concentrations remained liquefied after the 24-hour period.

A cumulative study was conducted with samples that did form a gel consistency to analyze the release kinetics of methylene blue. At day 0, 1 mL of phosphate buffered saline was added to each vial. Each day of the study, 1 mL of this phosphate buffered saline and methylene blue solution was removed and absorbance and concentration was measured using ultraviolet-visible spectroscopy. This mixture was then be discarded and new phosphate buffered saline was added to each sample until the gel had completely degraded. Samples of Pluronic F127 at 19 percent weight and above with 7.8% methylene blue proved to have the greatest longevity.

A second trial was conducted using 19-21 percent weight Pluronic F127 with 7.8% methylene blue in 10 mL and 20 mL vials to compare the effects of surface degradation. In comparison to the 20 mL vial, the 10 mL vial showed significant increase in longevity. Increased concentrations of Pluronic F127

Of the samples analyzed, 21 percent weight Pluronic F127 and 7.8% methylene blue proved to be the most clinically favorable sample for long-term delivery. Gel formation occurred instantly at room temperature and degraded over a period of 7 days. This specific sample also showed to have the highest percentage of methylene blue released during the cumulative release study.

Introduction

While the creation of hydrogels originated in 1894, they made their first appearance in the medical field in 1961, with the creation of soft contact lenses. They have consistently been a popular topic within the biomedical research community because of their biocompatible characteristics. Within the medical community today, they have commonly been used for wound therapy as they can mimic tissues and provide moisture to promote cell turnover (Wheeler, et al., 1996). However, more recently they have also shown to be potential drug vehicles capable of providing localized delivery. As compared to oral or intravenous delivery, this treatment method is novel in its ability to decrease the likelihood of autoimmune responses (Milling, Zhang, & Irvine, 2017).

Hydrogels are composed of a sophisticated hydrophilic matrix of polymers. Natural or synthetic polymers can be used in order to achieve an appropriate level of water absorption and longevity. These polymers are considered smart materials as they are capable of detecting and responding to changes in their physical and chemical environment (Ahmed, 2013).

The objective of my research is to determine the most biocompatible composition of a hydrogel composed of Pluronic F127 and Methylene Blue. This will be determined by conducting a cumulative release study using ultraviolet-visible spectroscopy. I chose to analyze potential medical benefits of this localized drug delivery in veterinary medicine, specifically pertaining to methemoglobinemia in small animals.

Literature Review

A 1% solution of methylene blue is typically administered intravenously to treat for methemoglobanemia in both humans and animals. Dosage for dogs and cats ranges from 1-10mg/kg over the span of several minutes (Constable, Hinchcliff, Done, Grünberg, & Radostits, 2017). Under normal circumstances, blood gas levels in small animals consists of 0-1% methemoglobin. Typically, oxidation of hemoglobin and reduction of methemoglobin occurs spontaneously each day to maintain homeostasis. However, methemoglobinemia can occur when the ferrous irons of the heme group in hemoglobin are oxidized from Fe^{2+} to Fe^{3+} and can no longer bind to oxygen molecules. An increase in as little as 10% of blood gas levels of methemoglobin can prevent oxygen from being released, leading to respiratory distress. At or above 20% methemoglobin levels are considered life threatening and patients can suffer from shock, seizures, and central cyanosis (Pharmocodynamics, n.d.).

Methemoglobinemia is typically acquired in small animals through the ingestion of toxins, specifically NSAIDS such as acetaminophen. Because of the widespread usage of NSAIDS, toxicosis is common is small animals. Within dogs, acute toxicosis can occur after ingestion of 100 mg/kg of acetaminophen. However, patients may not display clinical signs until 200 mg/kg is ingested. Cats can display clinical signs of cyanosis of mucous membranes at 10-40 mg/kg. Methemoglobanemia can also be induced by the use of localized anesthetics such as lidocaine and benzocaine (Patton, Blamer, & Horak, 2016).

An autosomal-recessive form can occur in which enzymatic reduction of methemoglobin is depleted. Type I of this congenital form has been documented in dogs and results from a mutation in Cytochrome B5 Type A. Affected patients may display cyanosis, hypoxia, disturbed or absent pubertal development, and undermasculinization. Serum testing will display elevated

levels of methemoglobin and sex steroid deficiency, decreased levels of erythrocyte cytochrome b5, and normal levels of glucocorticoids and mineralocorticoids. Type II is characterized by a complete loss of enzymatic activity of cytochrome b5 reductase which eventually leads to encephalopathy, loss of motor skills, and decreased life expectancy. However, type II has not been documented in small animals (McKenna, et al., 2014).

Methylene Blue is typically used for treatment in patients with either congenital or acquired forms of methemoglobinemia. It acts as a cofactor to reduced nicotinamide adenine dinucleotide phosphate (NADPH) as it is reduced by NADPH to leukomethylene blue to in turn become an electron donor and non-enzymatically reduce methemoglobin to hemoglobin. Methylene Blue has the potential to increase the NADPH methemoglobin reductase pathway roughly 5-to-10 fold.

However, when administered intravenously, methylene blue can lead to hemolytic anemia in patients with a glucose-6-phosphate dehydrogenase deficiency as it is an imperative enzyme in the formation of NADPH and low levels prevent function of the NADPH-dependent methemoglobin reductase pathway. Research of localized delivery of methylene blue in patients with G6PD deficiencies has not yet been conducted (Patton, Blamer, & Horak, 2016). The formation of Heinz bodies can be induced in cats when treated long-term with methylene blue containing antiseptics. However, when administered intravenously at reoccurring doses for nitrite-induced methemoglobinemia, there was no evidence to show that methylene blue caused Heinz body formation (Rumbeiha & Oehme, 1992).

Pluronic F-127, a poloxamer, is composed of a hydrophobic polyoxypropylene chain and two chains of hydrophilic chains of polyoxyethylene. In aqueous solutions, it assembles into micelles that are dependent on polymer concentration and temperature. An important property of

Pluronic F127 is its ability to be thermoreversible. Below critical micelle temperature, the tri-block of polymers becomes hydrated and soluble. This critical micelle temperature occurs near 37 degrees Celsius, making it physiologically compatible. Above this critical temperature, it will form a gel consistency and begin degradation. Pluronic F127 has surfactant properties that allow it to interact with hydrophobic substances and biological membranes (Nie, Hsiao, Pan, & Yang, 2011). In combination with water, it has been successfully used for drug and gene delivery as well as tissue scaffolding in stem cells and lung tissue (Cidade et al., 2019).

Materials and Methods

Aim 1: Fabrication and Characterization of the Injectable Hydrogel Platform.

Rationale: To prepare the hydrogel, Pluronic F127 was added to deionized water in solutions of 15-21 weight percent in 20 mL vials. The Pluronic F127 polymer was allowed to dissolve under mechanical stirring on a magnetic stir plate for 24 hours. Once this was complete, methylene blue was added in varying concentrations of 7.8, 15.6, and 31.2 weight percent to evaluate the release kinetics of different loading dosages. Each sample was then placed in an oven at 37 degrees Celsius. Gelation time was determined by the amount of time it took for the solution to no longer flow in the tube without reaching solidification.

Aim 2: Evaluate Release Kinetics of Pluronic F127-Injectable Hydrogel System

Rationale: Sample solutions were prepared as previously described with one caveat. Release studies were conducted by measuring absorbance spectroscopy of Methylene Blue. The absorbance curve generated by these trials were compared with a baseline absorbance curve from Methylene Blue solution. The most efficient absorbance indicated the concentration of Methylene Blue needed. Once this concentration was achieved, cumulative release studies were conducted to determine release time in 24-hour time intervals.

Aim 3: Modification of Injectable Hydrogel and Re-evaluation of Release Kinetics Based on Vial Volume

Rationale: Based on the results from previous methodology, a specific concentration of Pluronic F127 and methylene blue was chosen to evaluate the impact of vial volume on surface degradation and release kinetics. This was conducted by creating hydrogels as previously described within two different vial sizes of 10 mL and 20 mL. Concentrations of Pluronic F127 and methylene blue

were determined by longevity and absorbance values from original trials. Release studies were again conducted using ultraviolet-visible spectroscopy.

Data Analysis

Figure 1. 7.8% Methylene Blue in 20 mL Vial

Percent Weight Pluronic F127	Gel Time	Day 1 Absorbance	Day 1 Concentration	Day 2 Absorbance	Day 2 Concentration	Day 3 Absorbance	Day 3 Concentration
5%	No gel formation	-	-	-	-	-	-
10%	No gel formation	-	-	-	-	-	-
15%	No gel Formation	-	-	-	-	-	-
20%	Gels instantly at room temperature.	1.113 1 mL of hydrogel solution	0.73	1.143 0.75 mL hydrogel solution with 2 mL added PBS	0.75	0.580 0.5 mL hydrogel solution with 2 mL PBS	0.38

Gel formation did not occur for 5-15% weight Pluronic F127. Gel formation did occur at 20% weight Pluronic F127 but an ice bath was needed to continue stirring the polymer as gel formation occurred immediately at room temperature. The ice bath decreased the temperature of the solution which allowed the magnetic stir bar to move freely. Complete degradation of the 20% weight gel occurred after three days.

Figure 2. 15.6% Methylene Blue in 20 mL Vial

Percent Weight Pluronic F127	Gel Time	Day 1 Absorbance	Day 1 Concentration
15%	No gel formation	-	-
16%	No gel formation	-	-
17%	Gel formation after 24 hours	1.349	0.89 0.5 mL hydrogel solution with 1.5 mL PBS added
18%	Gel formation after 4 minutes	1.007	0.66 0.5 mL hydrogel solution with 1.5 mL PBS added

Gel formation did not occur at 15% or 16% Pluronic F127. While gel formation did occur after 24 hours for 17%, this would not be a favorable gel time for clinical use. Both 17% and 18% degraded after a single day.

Figure 3. 31.2% Methylene Blue in 20 mL Vial

Percent Weight Pluronic F127	Gel Time	Day 1 Absorbance	Day 1 Concentration
15%	No gel formation	-	-
16%	No gel formation	-	-
17%	Gel at 24 hours	1.341	0.88 0.5 mL hydrogel solution and 2.5 mL PBS added
18%	Gel at 7 minutes	1.359	0.5 mL hydrogel solution and 3 mL PBS added

Gel formation occurred at 17% and 18% Pluronic F127. 18% would be more clinically relevant because of the shorter gel time of 7 minutes. However, both concentrations completely degraded after one day.

Higher concentration of 20 percent weight Pluronic F127 and 7.8% Methylene Blue showed slower release kinetics, so a new trial using 19, 20, and 21 percent weight Pluronic F127 was conducted with 7.8% Methylene Blue. Two separate vial sizes of 10 mL and 20 mL were used to compare the impact of vial volume on surface degradation and release kinetics. For all samples, gel formation occurred instantly at room temperature. Samples with Pluronic F127

concentrations at or above 20 weight percent required an ice bath while stirring to prevent gel formation at room temperature.

Figure 4. Calibrations Curve of Methylene Blue

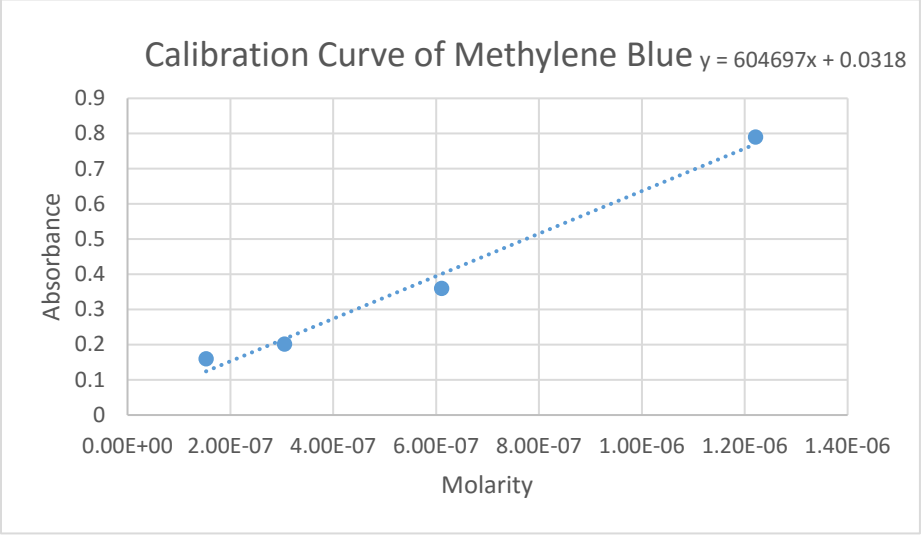


Figure 5. 19% Pluronic F127 and 7.8 % Methylene Blue in 20 mL Vial

Day	Absorbance	Concentration
1	1.000	0.66 1 mL hydrogel solution and 2 mL PBS added
2	0.910	0.60 1 mL hydrogel solution and 2 mL PBS added

Figure 6. Cumulative Release of 7.8% Methylene Blue with 19% Pluronic F127 in 20 mL Vial

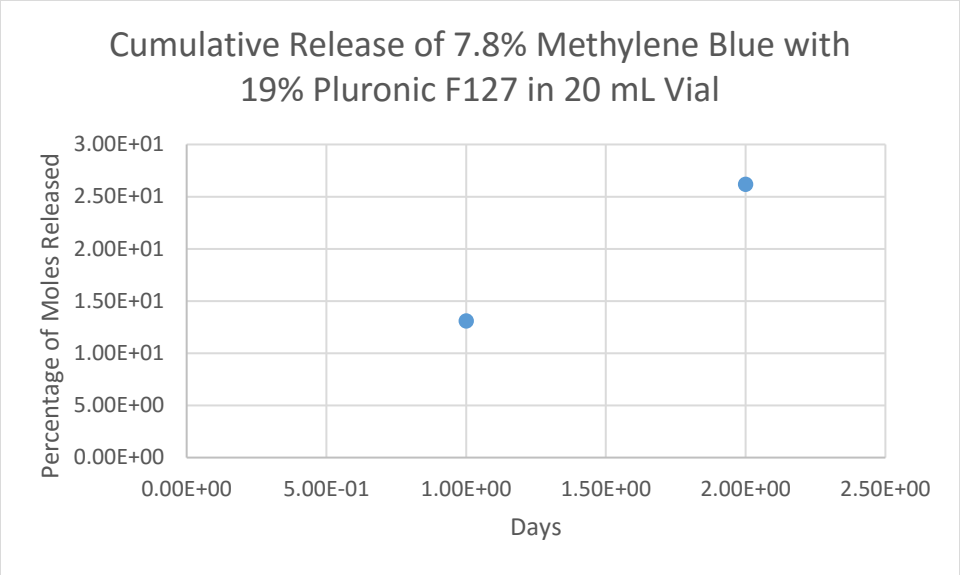


Figure 7. 20% Pluronic F127 and 7.8 % Methylene Blue in 20 mL Vial

Day	Absorbance	Concentration
1	0.798	0.53 1 mL hydrogel solution and 4 mL PBS added
2	0.981	0.65 1 mL hydrogel solution and 5 mL PBS added

Figure 8. Cumulative Release of 7.8% Methylene Blue with 20% Pluronic F127 in 20 mL Vial

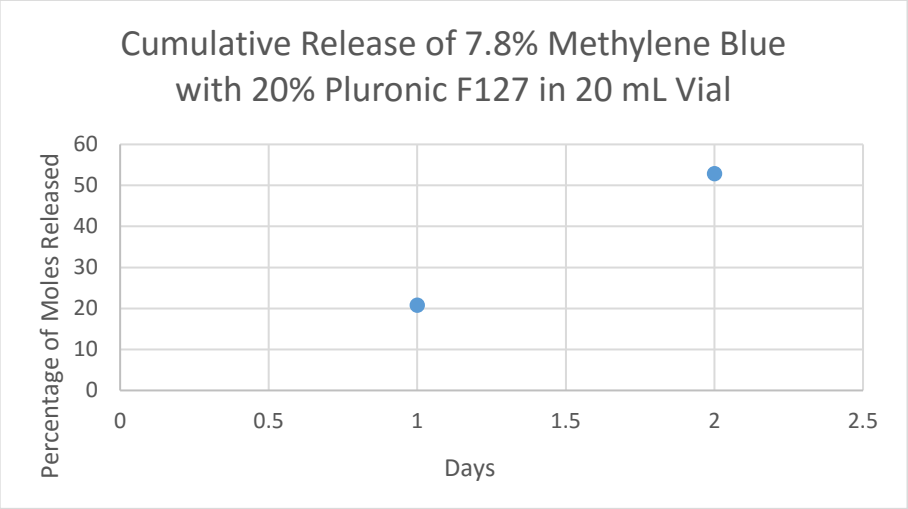


Figure 9. 21% Pluronic F127 and 7.8 % Methylene Blue in 20 mL Vial

Day	Absorbance	Concentration
1	0.398	0.26 1 mL hydrogel solution and 3 mL PBS added
2	0.810	0.53 1 mL hydrogel solution and 3 mL PBS added

Figure 10. Cumulative Release of 7.8% Methylene Blue with 21% Pluronic F127 in 20 mL Vial

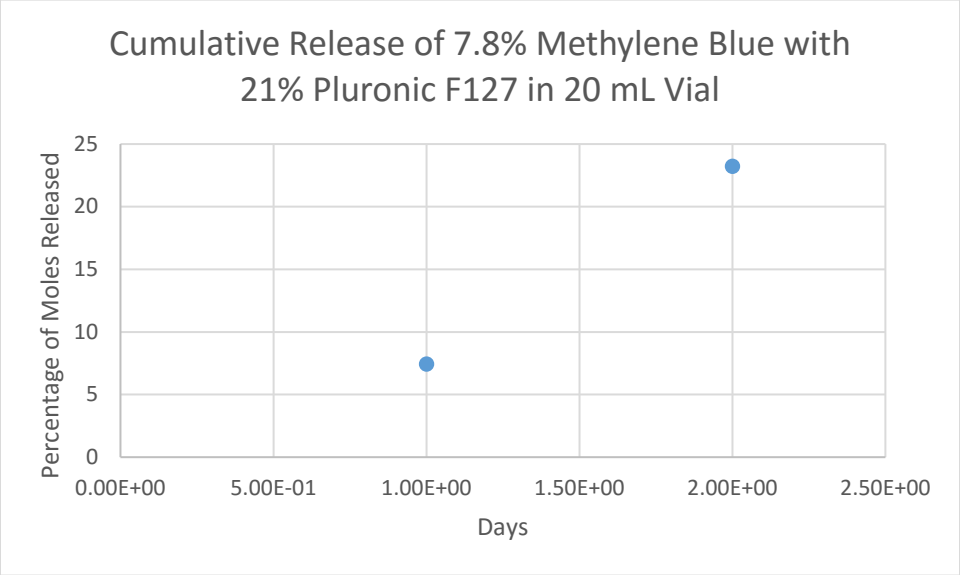


Figure 11. 19% Pluronic F127 and 7.8% Methylene Blue in 10 mL Vial

Day	Absorbance	Concentration
1	0.971	0.64 1 mL hydrogel solution with 1 mL PBS added
2	0.916	0.60 1 mL hydrogel solution with 2 mL PBS added
3	0.752	0.50 1 mL hydrogel solution with 1 mL PBS added
4	0.771	0.51

		1 mL hydrogel solution with 2 mL PBS added
5	0.697	0.46 1 mL hydrogel solution with 2 mL PBS added
6	0.933	0.62 1 mL hydrogel solution with 1 mL PBS added
7	0.768	0.51 1 mL hydrogel solution with 2 mL PBS added

Figure 12. Cumulative Release of 7.8% Methylene Blue with 19% Pluronic F127 in 10 mL Vial

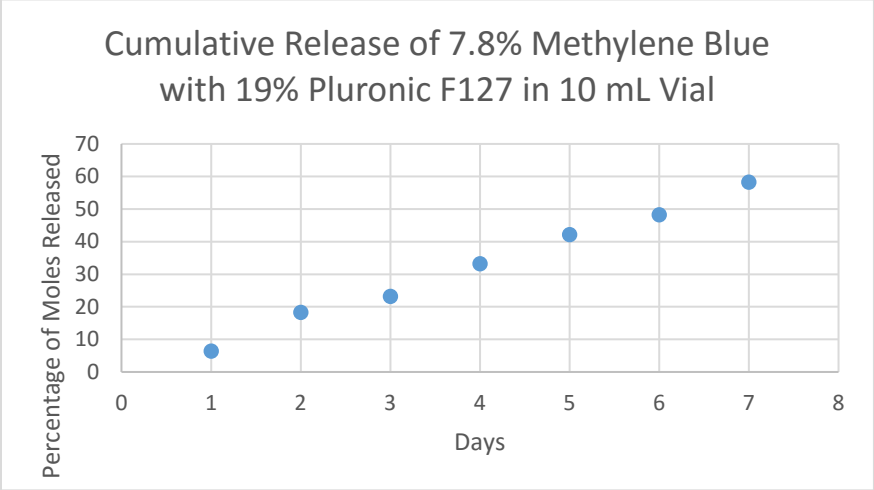


Figure 13. 20% Pluronic F127 and 7.8% Methylene Blue in 10 mL Vial

Day	Absorbance	Concentration
1	0.672	0.44 1 mL of hydrogel solution with 2 mL PBS added
2	0.957	0.63 1 mL of hydrogel solution with 2 mL PBS added
3	0.786	0.52 1 mL of hydrogel solution with 2 mL PBS added
4	0.838	0.55 1 mL hydrogel solution with 1 mL PBS added
5	0.960	0.63 1 mL hydrogel solution with 1 mL PBS added
6	0.505	0.33 1 mL hydrogel solution with 2 mL PBS added

Figure 14. Cumulative Release of 7.8% Methylene Blue with 20% Pluronic F127 in 10 mL Vial

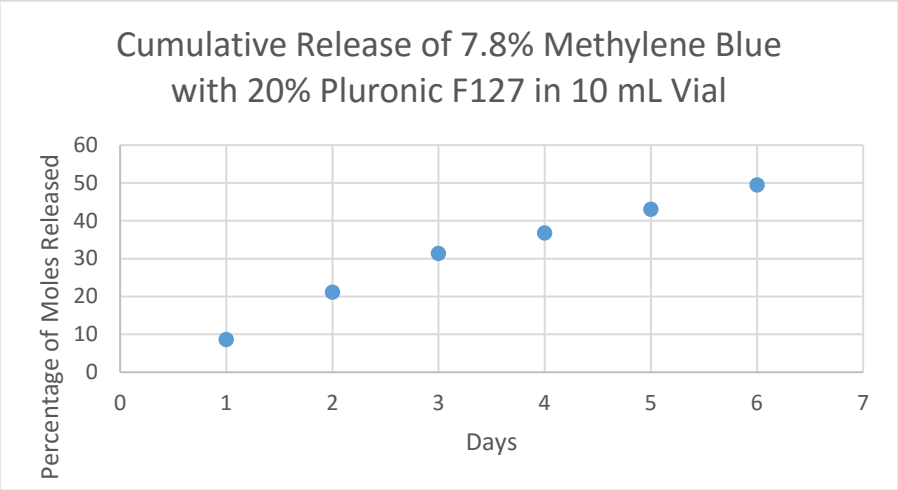
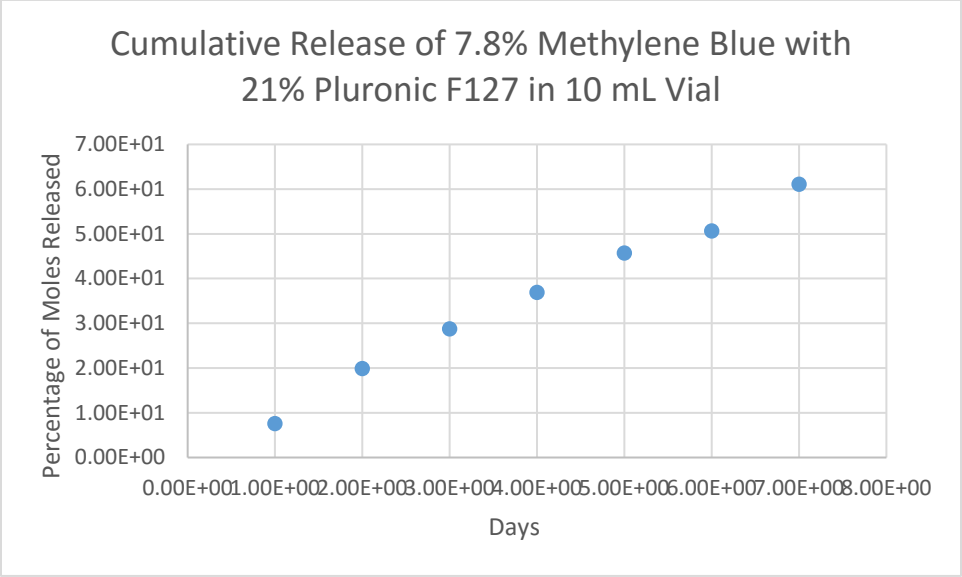


Figure 15. 21% Pluronic F127 with 7.8% Methylene Blue in 10 mL Vial

Day	Absorbance	Concentration
1	0.592	0.39 1 mL hydrogel solution with 4 mL PBS added
2	0.941	0.62 1 mL hydrogel solution with 2 mL PBS added
3	0.685	0.45 1 mL hydrogel solution with 2 mL PBS added
4	0.638	0.42 1 mL hydrogel solution with 2 mL PBS added
5	0.680	0.45 1 mL hydrogel solution with 2 mL PBS added
6	0.765	0.50 1 mL hydrogel solution with 1 mL PBS added
7	0.802	0.53 1 mL hydrogel solution with 2 mL PBS added

Figure 16. Cumulative Release of 7.8% Methylene Blue with 21% Pluronic F127 in 10 mL Vial



Discussion

All three solutions within the large vials degraded within two days, making them more useful for short-term medical use. The 10 mL vials proved more relevant for long-term release as they degraded in the span of 6-7 days. 21% Pluronic F127 with 7.8% methylene blue in a 10 mL vial proved to be the most appropriate combination for long-term release as its degradation period was the longest and percentage of methylene blue released was the highest at 61.1%. For short-term use, 20% Pluronic F127 with 7.8% methylene blue in a 20 mLvial would be the most appropriate as it released the highest percentage of methylene blue at 52.8%. Degradation of the hydrogels was influenced by the volume of the vial in which they were created. Varying concentrations of methylene blue showed to be insignificant in altering surface degradation time and absorption. Manipulation of these variables was more influenced by vial size and polymer concentration. For long-term use as in the case of congenital patients, Pluronic F127 may not be a suitable polymer as concentrations above 21% may be difficult to solubilize.

Conclusions and Implications

Having a single injection of the methylene blue hydrogel would make treatment more feasible for patients requiring long-term treatment rather than maintaining a consistent intravenous method. Concentrations of both methylene blue and Pluronic F127 can be easily manipulated in order to achieve appropriate dosage and longevity for the specific patient. However, weight percentages of Pluronic F127 at 19%-21% would likely be a favorable concentration in a clinical setting as gel formation occurs instantly at body temperature. Localized delivery of methylene blue might also create potential for patients suffering from methemoglobanemia that also have a glucose-6-phosphate dehydrogenase deficiency as smaller amounts of NADPH might be efficient. Methylene blue hydrogels might also be more beneficial for cats as they're more susceptible to Heinz body formation and hemolytic anemia. Delivering long-term, low doses could potentially reverse the effects of methemoglobinemia without inducing life-threatening side-effects.

Moving forward, animal trials would be most appropriately conducted in large animals such as cattle and sheep as methylene blue is more commonly used for toxicosis. Future treatment in small animals may also be more financially effective as minimal amounts of Pluronic F127 and methylene blue are needed. Hospitalization and IV catheter placement would also no longer be necessary. Because of the thermoreversible properties of Pluronic F127, hydrogels could be prepared ahead of time and stored in a refrigerator at 3 degrees Celsius without gel formation or surface degradation. Upon being introduced into the body, the hydrogel solution would instantly form a gel consistency and begin release of methylene blue.

Further analysis of different injection sites could also be conducted in the future to manipulate the rate of absorption of methylene blue. Because of its biocompatible nature, a hydrogel injectable isn't isolated to subcutaneous delivery.

References

Ahmed, E. M. (2013, July 18). Hydrogel: Preparation, characterization, and applications: A review. Retrieved from https://www.sciencedirect.com/science/article/pii/S2090123213000969#b0065

Cidade M. T., Ramos, D. J., Santos, J., , H., Calero, N., & Borges, J. P. (2019, April 02). Injectable Hydrogels Based on Pluronic/Water Systems Filled with Alginate Microparticles for Biomedical Applications. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6479463/

Constable, P. D., Hinchcliff, K. W., Done, S. H., Grünberg, W., & Radostits, O. M. (2017). *Veterinary medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats* (11th ed.).

Khan, S. A., ASPCA Animal Poison Control Center, Urbana, Illinois. (n.d.). Analgesics (Toxicity) - Toxicology. Retrieved from https://www.merckvetmanual.com/toxicology/toxicities-from-human-drugs/analgesics-toxicity

McKenna, J. A., Sacco, J., Son, T. T., Trepanier, L. A., Callan, M. B., Harvey, J. W., & Arndt, J. W. (2014). Congenital methemoglobinemia in a dog with a promoter deletion and a nonsynonymous coding variant in the gene encoding cytochrome b_5. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4895586/#jvim12423-bib-0001

Methemoglobanemia: Treatment. (n.d.) Retrieved from

https://www.openanesthesia.org/methemoglobanemia

Metheylene Blue Pathway, Pharmocodynamics. (n.d.) Retrieved from

https://www.pharmgkb.org/pathway/PA165980834

Milling, L., Zhang, Y., & Irvine, D. J. (2017, May 15). Delivering safer immunotherapies for cancer. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5647831/

Nie, S., Hsiao, W. L., Pan, W., & Yang, Z. (2011). Thermoreversible Pluronic F127-based hydrogel containing liposomes for the controlled delivery of paclitaxel: In vitro drug release, cell cytotoxicity, and uptake studies. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3075891/

Patton, T. G., Blamer, S. L., & Horak, K. E. (2016, December 08). Detecting methemoglobinemia in animals with a drop of blood. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5145221/

Pluronic. (n.d.). Retrieved from https://www.sciencedirect.com/topics/chemistry/pluronic

Pluronic F127 Hydrogel characterization and biofabrication in cellularized constructs for tissue engineering applications. (2016, July 28).

Richardson, J. A., DVM. (2000, July). Management of acetaminophen and ibuprofen toxicoses in dogs and cats.

Rumbeiha, W. K., & Oehme, F. W. (1992, April). Methylene blue can be used to treat methemoglobinemia in cats without inducing heinz body hemolytic anemia. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1509670

Wheeler, J. C., Woods, J. A., Cox, M. J., Cantrell, R. W., Watkins, F. H., & Edlich, R. F. (1996). Evolution of hydrogel polymers as contact lenses, surface coatings, dressings, and drug delivery systems. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/10167362

Zhou, Y., Boudreau, D. M., & Freedman, A. N. (2014, January). Trends in the use of aspirin and nonsteroidal anti-inflammatory drugs in the general U.S. population. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/23723142