Carprofen Delivery Via Water-Based Gel

A Study To Determine Drug Homogeneity, Stability and Gel Sterility

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ABSTRACT

Pain management for research animals is a scientific and moral imperative. Appropriate use and selection of an analgesic minimizes the confounding variables of pain in a study and mitigates the discomfort experienced by the animal. The nonsteroidal anti-inflammatory drug (NSAID) Carprofen is widely used in an injectable formulation, though this route requires daily restraint and injection in mice leading to additional distress/discomfort on the recovering animal as well as spikes in blood plasma levels. Additionally, there is increased risk of skipped injections and added labor costs. Until late 2019 a commercial diet gel formulation containing Carprofen was available that assisted in overcoming some of the negative aspects of injectable Carprofen. Unfortunately, this product was removed from the market due to regulatory hurdles. Having experienced great analgesic success and researcher compliance with this product we sought to replicate the oral delivery of Carprofen using a sucralose-sweetened gel. To this end, a process for mixing and storing the in-house Carprofen gel was tested with the intent of determining homogeneity of the mixing process. Sterility and stability following the addition of Carprofen to gel cups, were analyzed over time (2 cups/time point/storage method). It was determined that a combination of mixing modes (hand agitation and vortexing) produced a homogeneous mixture. Following inoculation of the Carprofen into the sealed gel cup the sterility of the gel was maintained for up to 6 mo. High performance liquid chromatography results demonstrated that the Carprofen distribution was homogeneous and remained stable at therapeutic concentrations for up to 3 mo (room temp) and 6 mo (refrigerated). In summary, this research demonstrates the relative ease with which an in-house gel-based delivery of Carprofen can be achieved while maintaining sterility and stability of the analgesic Carprofen.

INTRODUCTION

The use of animals in biomedical research requires ample consideration of the best methods to identify and alleviate pain/discomfort.[1] The research community has a scientific and moral obligation to ensure that pain is mitigated while simultaneously striving to deliver data that are relevant and reproducible.[2] It is not possible to eliminate procedures that cause discomfort in rodent models, but adequate analgesic regimens can help to counteract the negative effects of pain. The NSAID Carprofen is a COX inhibitor which diminishes inflammation and pain in rodents and other species.[3] It can be administered orally or via subcutaneous injection to treat mild pain or can be part of a multimodal strategy for more invasive procedures. One advantage of voluntary oral administration of Carprofen is that the animal does not need to be restrained as the drug can be added to a hydration source in the cage.[4] Our program was successfully using a commercially available Carprofen-infused diet/hydration gel product until the latter part of 2019 when the product was discontinued. Not wanting to abandon a beneficial drug and delivery mechanism, we sought to make our own Carprofen gel cups with a different commercially available gel (MediGel® Sucralose). Prior to implementing this in-house approach, it was important to determine the homogeneity and stability of the Carprofen as well as the sterility of the gel after inoculation with the Carprofen solution.

Figure 1. Gel in unopened cup was melted in a water bath (50-60 C°) and inoculated with dyed solution after wiping the lid with alcohol. The puncture in the lid was then covered with a sticker and the cup was dated.

METHODS

- Visual homogeneity Test: Gel melted in sealed cup inside 50 C° water bath until liquid. Three volumes of sterile food dye (100 ul, 250 ul, and 500 ul) were injected into melted gel followed by vigorous hand-agitation for 10 seconds. (Figures 1 and 2).
- Vortexing was added to the procedure to determine whether this would enhance the hand-agitation only mixing. For this test gel was melted in water bath then inoculated with a mixture of dye and Carprofen to achieve a concentration of 23-25 mg/kg. Re-Solidified gels were stored and shipped either refrigerated or at ambient temperature before being analyzed using HPLC 48 hours later.
- Vortex-shaken Carprofen + dye cups were generated and stored (refrigerated and ambient temperature) 7, 14, 28, 35, 90, and 180 days at which point the gels were tested for sterility (aerobic culture) and Carprofen concentration (HPLC).

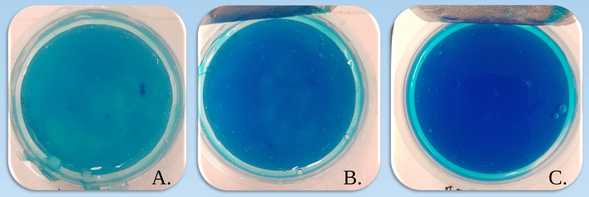

Figure 2. Visual homogeneity test. Each melted gel cup received either 100 ul. (A.), 250 ul. (B.), or 500 ul. (C.) of sterile dye solution followed by hand agitation.

RESULTS

- The visual homogeneity test demonstrated that 100 ul of dye solution was optimal because it was dilute enough to allow for visualization of unmixed dye. (Figure 2)
- Carprofen homogeneity test for vortexed and non-vortexed samples revealed that vortexing resulted in a more homogeneous and consistent Carprofen mixture compared to hand mixing alone. (Figure 3)
- Aside from one positive culture (day 7) all gel cups remained sterile following inoculation of Carprofen solution through the foil lid. (Table 1)
- Concentration of Carprofen remained stable for up to 180 days, with refrigeration appearing to provide some benefit at later time points. Concentration comparable to previous commercial product. (Figure 4)

Sample Day	Refrigerated	Room Temperature
Day 7	Negative	1 Positive
Day 14	Negative	Negative
Day 28	Negative	Negative
Day 35	Negative	Negative
Day 90	Negative	Negative
Day 180	Negative	Negative

Table 1. Gel Sterility Test. "Sample Day" indicates the number of days post-inoculation with Carprofen solution. One positive culture was *Corynebacterium lipophiloflavum* (likely contamination at sampling time). One cup sampled per time point and storage temperature.

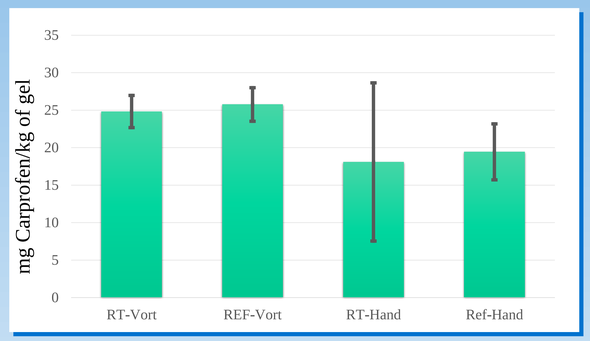

Figure 3. Carprofen homogeneity in Gel. Both refrigerated (REF) and ambient temperature (RT) samples that were vortexed following gel melting and Carprofen inoculation were more consistent than hand agitation alone (Hand). Measurements taken 48 hours after mixture.

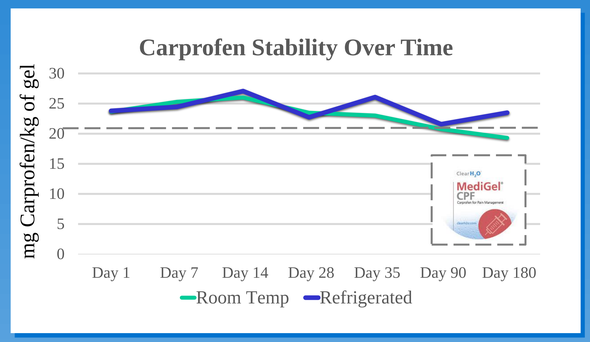

Figure 4. Carprofen stability test. Both refrigerated (REF) and ambient temperature (RT) samples that were vortexed following gel melting and caroprofen inoculation were more consistent than hand agitation alone (Hand). Grey dashed line represents concentration of previous commercial product.

CONCLUSION

- The NSAID Carprofen can be easily added to a thermoreversible gel by melting the gel at 50-60 C° and puncturing the foil lid with a needle. Homogeneity of Carprofen best achieved with vortexing of melted gel.
- Sterility of the gel cup was maintained for up to 180 days (ambient or refrigerated temperatures) by covering the puncture hole with a sticker.
- Stability of Carprofen observed for up to 180 days on par with the previous commercial Carprofen product. This would allow for large batches of gel cups to be made up in advance of their need.
- Future work: Studies of blood plasma levels in a surgical model together with behavioral metrics and/or consumption studies would give a more complete picture of the true benefits of this Carprofen delivery method.

REFERENCES

1. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. *Guide for the Care and Use of Laboratory Animals*. 8th ed. National Academies Press (US); 2011. Accessed September 26, 2020. http://www.ncbi.nlm.nih.gov/books/NBK54050/
2. National Research Council (US) Committee on Recognition and Alleviation of Pain in Laboratory Animals. *Recognition and Alleviation of Pain in Laboratory Animals*. National Academies Press (US); 2009. Accessed September 26, 2020. http://www.ncbi.nlm.nih.gov/books/NBK32658/
3. Adamson TW, Kendall LV, Goss S, et al. Assessment of carprofen and buprenorphine on recovery of mice after surgical removal of the mammary fat pad. *Journal of the American Association for Laboratory Animal Science: JAALAS*. 2010;49(5):610-616.
4. Turner PV, Brabb T, Pekow C, Vasbinder MA. Administration of substances to laboratory animals: routes of administration and factors to consider. *J Am Assoc Lab Anim Sci*. 2011;50(5):600-613.

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