Preliminary Investigation on Reducing Ocular Irritation Potential of Harsh Ingredients By Increasing Formulation Viscosity

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ABSTRACT

Formulations tested for ocular irritation using the Bovine Corneal Opacity and Permeability (BCOP) assay may be assigned a specific irritation label based on the resulting *In Vitro* Irritancy Score (IVIS) and specific regulatory guidelines (e.g. OECD, EPA, and CLP) that provide cutoff values for classifications. The ability to reduce ocular irritation by slightly adjusting the physical properties of a formulation is highly desirable. Laboratory investigations found that incrementally changing viscosity using increasing amounts of Carbopol® as a thickening agent reduced ocular irritation when mixed with a 1% NaOH solution in water. Following a 10-minute exposure in the BCOP assay, 1% NaOH was previously classified as a severe ocular irritant (IVIS=161.6). Increasing Carbopol® from 0.25% to 1.25% in a mixture with 1% NaOH decreased the *In Vitro* score to a range of values between 150.9 and 18.3 and decreased ocular irritation across a range of irritation classifications from severe to mild irritation (n= 3 corneas per treatment). Exposure to 1% Carbopol® alone exhibits minimal irritation (IVIS=1.3) and Carbopol® is consequently not considered to contribute to ocular irritation within the tested mixtures. Histopathology evaluation further supports that exposure to 1% Carbopol® results in damage similar to negative control treated corneas and that epithelial and stromal damage decreases as viscosity increases. Additionally, preliminary findings indicate that when a small amount of thickener is added to a complex formulation containing otherwise harsh ingredients, ocular irritation can be mitigated from a Category I label to a Category II label according to current EPA guidelines applicable to cleaning products making anti-microbial claims. Similarly, increasing the viscosity of a formulation containing more than 3% of a severe ingredient also resulted in a "Not Classified" label according to OECD criteria when it would have received a severe classification if left untested (according to CLP regulations). These results indicate that increasing viscosity may be an effective tool for reducing ocular irritation potential of a formulation. Viscosity, among other physical properties, may therefore be used to inform decision making during product development, ultimately affecting downstream users in such areas as marketing, labeling, packaging and distribution.

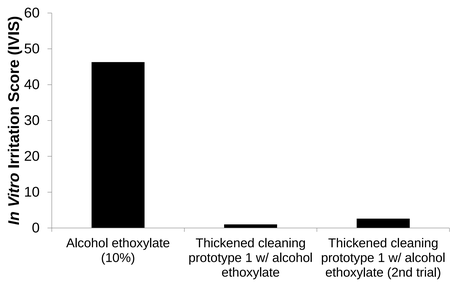
INTRODUCTION

Formulations tested for ocular irritation using the BCOP assay may be assigned a specific irritation label based on the resulting *In Vitro* score and specific regulatory guidelines (e.g. OECD, EPA, and CLP) that provide cutoff values for classifications. The ability to reduce ocular irritation by slightly adjusting the physical properties of a formulation is highly desirable.

According to current EPA guidelines (31 May 2013), substances tested in the BCOP assay resulting in an IVIS ≥ 75 would receive a Category 1 classification indicative of irreversible ocular damage, and those resulting in an IVIS < 75 would receive a Category II classification indicating substantial but temporary ocular injury. OECD test guideline 437 (26 July 2013) classifies test substances with an IVIS > 55 as Category 1, indicating severe irritation or corrosive eye damage.

EPA Use of BCOP	OECD / GHS Use of BCOP
IVIS ≥ 75 = Cat I	IVIS > 55 = Cat 1
IVIS < 75 = Cat II	IVIS ≤ 3 = Not Classified
Not used for Cat III or IV prediction	IVIS> 3 but ≤ 55 = No prediction; perform another test

Initial lab testing using alcohol ethoxylate (a common surfactant ingredient labeled as a Category 1 substance by the supplier) indicated increasing viscosity reduces ocular irritation. According to EU Classification, Labeling and Packaging (CLP) regulations, a mixture containing a ≥ 3% concentration of alcohol ethoxylate would receive a Category 1 label, unless otherwise tested. When thickened, a tested cleaning prototype formulation with > 3% of the alcohol ethoxylate met the criteria for "No classification" (i.e., IVIS = 0-3).



OBJECTIVES

1) Test a common, harsh ingredient (1% NaOH) independently using the BCOP assay and compare results when the ingredient is combined with increasing amounts of a commonly used thickener (Carbopol®).

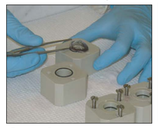
2) Add thickener to a complex formulation containing harsh ingredients and evaluate findings.

MATERIALS AND METHODS

Corneal Excision

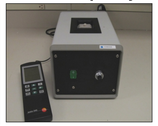
Step 1: Upon receipt, bovine eyes were visually examined and only the corneas without defects were excised.

Mounting



Step 2: Corneas were mounted into chambers and incubated for 1 hr at 32 ± 1°C in complete minimum essential medium (cMEM).

Initial Opacity

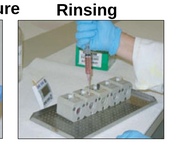


Step 3: cMEM was removed and refilled, and the initial corneal opacity was read using an opacitometer.

Test Article Exposure

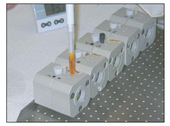
Step 4: 750 µL of test substance was applied to the epithelial side of the cornea (anterior chamber) for 10 min at 32 ± 1°C.

Rinsing



Step 5: Corneas were rinsed thoroughly to remove the test substance and incubated for 2 hrs before a final opacity reading.

Fluorescein Addition

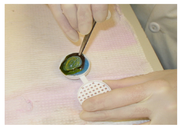


Step 6: 1 mL of a 4 mg/mL fluorescein solution was added to the epithelial side of the corneas and incubated at 32 ± 1°C for 90 min.

Permeability Endpoint

Step 7: Media was sampled from the posterior chamber, and the optical density at 490 nm was quantified via a microplate reader.

Fixing the Corneas



Step 8: Treated corneas were saved from the assay and fixed in formalin for histological evaluation.

***In Vitro* Irritation Score (IVIS) = Mean Opacity Value + (15 x Mean OD_{490})**

RESULTS

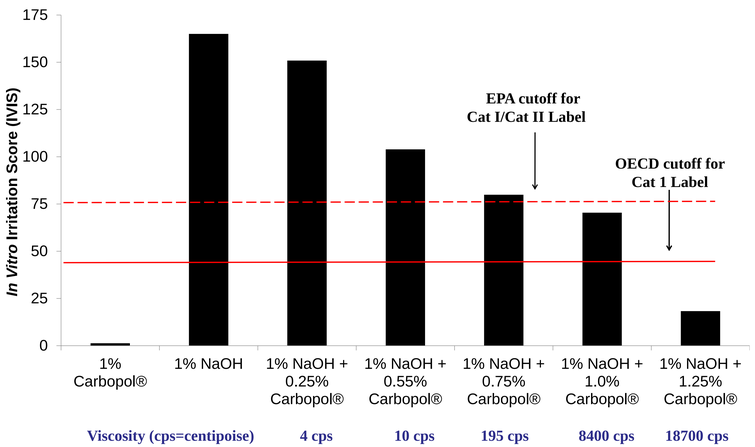


Figure 1: Ocular irritation of harsh ingredients diminishes as viscosity increases.
The BCOP assay (n=3 corneas per sample) was performed for 1% NaOH solutions thickened with an increasing amount of Carbopol® to modify the viscosity of the solution. The 1% Carbopol® control solution demonstrates that the Carbopol® is not contributing to the irritation, whereas the 1% NaOH positive control solution indicates that the solution is corrosive based on the BCOP classification criteria.

RESULTS (continued)

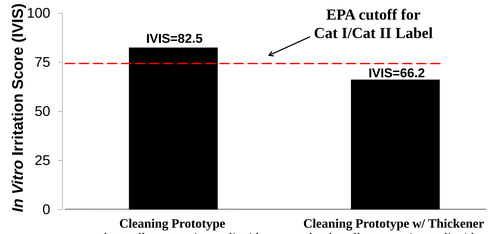


Figure 2: Altering the viscosity of a cleaning prototype complex formulation can also attenuate ocular irritation.
A cleaning prototype composed of surfactants and solvents was tested in the BCOP assay and met the EPA criteria for a Category I corrosive formula (IVIS=82.5). When the formula was thickened slightly (still remained a free-flowing liquid), the BCOP results were below the threshold for EPA Category I (IVIS=66.2).

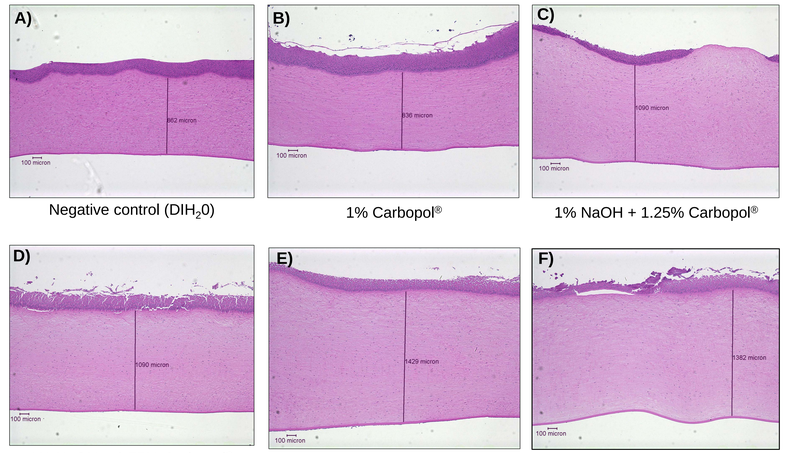


Figure 3. Histopathology Evaluation.
In 1% Carbopol® treated corneas, only upper epithelial damage was observed and stromal and endothelial observations were similar to the negative control treated corneas (A-B). As Carbopol® concentration decreased in the 1% NaOH mixture, an increase in the degree of damage to the corneas was observed as compared to Carbopol® only treated corneas (B-F); epithelial changes including sloughing and detachment from the basement membrane, denaturation of the epithelial proteins, and blanching of the nuclei in the epithelium were observed. The stroma and endothelium exhibited progressive damage with increasing stromal swelling and eventual loss of endothelial function (C-F).

CONCLUSIONS

- The BCOP assay is a rapid, effective non-animal method to evaluate the ocular irritation potential of cleaning formulations.
- Increasing viscosity may be an effective tool for reducing ocular irritation potential of a formulation. Viscosity, among other physical properties, may therefore be used to inform decision making during product development, ultimately affecting downstream users in such areas as marketing, labeling, packaging and distribution
- Histology is able to provide useful information regarding the degree and depth of injury including identifying specific changes in the epithelium, stroma, and endothelium.

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