Predicting Ocular Irritation of Surfactants Using the Bovine Corneal Opacity and Permeability Assay

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ABSTRACT

Background

The Bovine Corneal Opacity and Permeability (BCOP) assay is an *ex vivo* test used to evaluate ocular irritation. According to the OECD Test Guideline (TG) 437, the BCOP assay can be used to identify chemicals which induce severe/corrosive eye irritation and those that do not require classification. However, BCOP has historically under-predicted certain anionic surfactants, when tested according to the standard liquid protocol. TG 437 specifies that liquid and solid surfactants may be tested as 10% aqueous dilutions for 10 minutes (although alternate dilutions and exposure times may be conducted with scientific rationale). The relevant guidance document (GD) No. 160 suggests that solid and concentrated liquid surfactants may be diluted to 10% for testing. However, GD No. 160 further directs that surfactant-based formulations are usually tested neat, but could be diluted with justification, imparting some confusion in identifying the most appropriate test methods. Additionally, as part of the EPA classification of ocular irritation, the BCOP assay may be used to assess anti-microbial products with cleaning claims. Such products may contain surfactants and are generally tested neat for classification purposes.

Methods

Since neither the basis for selecting the appropriate surfactant test methods, nor the justification for modifications are clearly presented in TG 437 or GD No. 160, we present on the testing of a few common surfactant ingredients, including sodium lauryl sulfate (SLS), Triton X-100, and benzalkonium chloride, and surfactant based formulations in the BCOP assay using standard and modified dilutions and exposures to elucidate the impact of these variables on eye irritation prediction.

Results and Discussion

As examples, *in vitro* scores of 20.7, 28.4, and 28.3 were obtained when testing SLS at concentrations of 50, 20, and 10% for 10 minutes, showing that irritation responses were not fully concentration-dependent. As a complement to the BCOP assay, histopathology was performed to assess the surfactant-induced corneal changes. Based upon these results, a framework for testing surfactant ingredients and surfactant-based formulations is proposed.

INTRODUCTION

In this study we investigated the BCOP assay for evaluation of the ocular irritancy potential of surfactants. There are several key considerations when evaluating surfactants in the BCOP assay.

KEY CONSIDERATIONS:

- Is the sample to be tested for regulatory classification and labeling?
  If so, what is the appropriate regulatory protocol per OECD TG 437[1].
- Is the assay being conducted to support product development? Alternate protocols may be used to enhance resolution and rank ordering of prototypes.
- What are the physicochemical properties of the sample (liquid/solid, viscosity, charge, pH)
- Is the sample an ingredient or formulation
- What exposure conditions are being modeled (industrial hygiene, transport, end use)
- Is the sample for professional or home use
- Is the formulation a concentrate or at end-use strength
- If the sample is a formulation what are the other components that may contribute to irritation potential
- The fluorescein permeability value generated by the BCOP assay may be the most relevant endpoint, as opacity, and the subsequent In Vitro Score may be artificially low

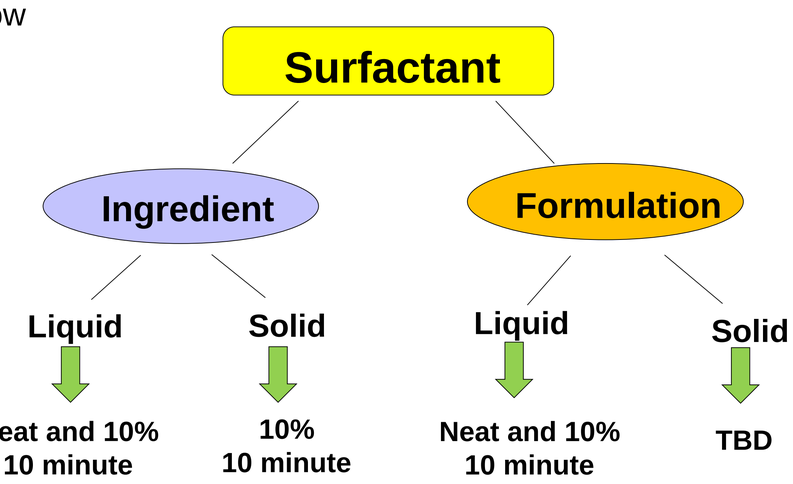
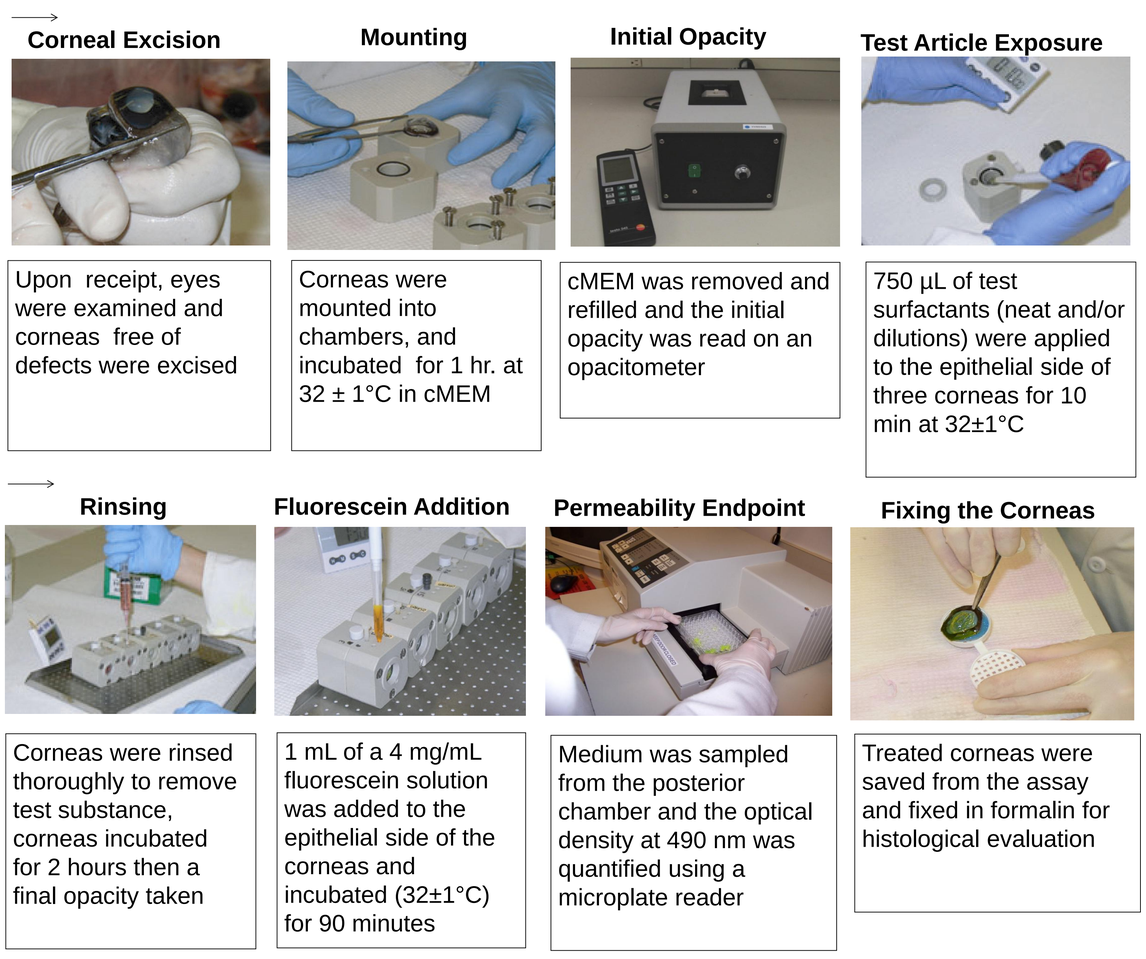


Figure 1. Decision tree for BCOP testing approach to surfactants. Protocols recommended for each type of sample (sample preparation- neat or diluted, and exposure time – 10 minutes). For solid formulations protocol should be determined based on the formulation components.

MATERIALS AND METHODS



RESULTS

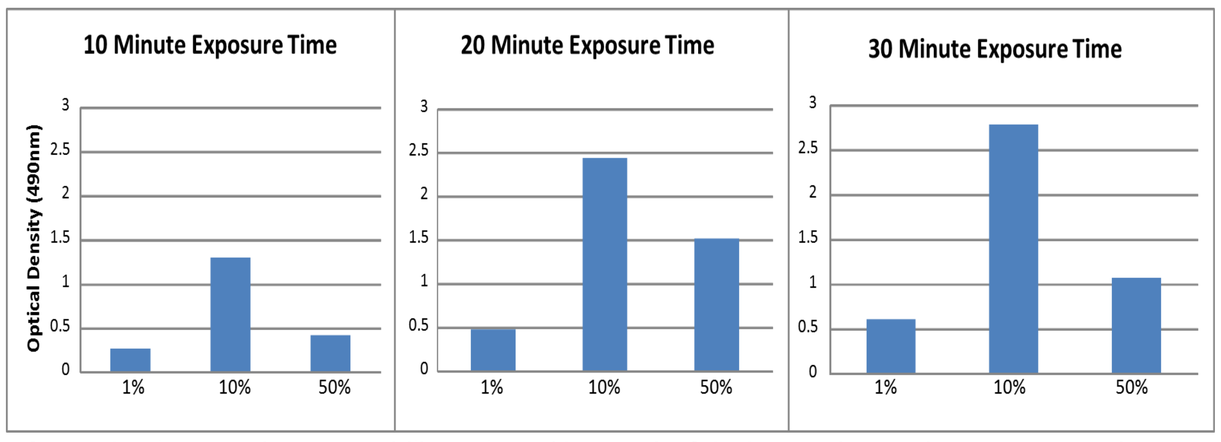
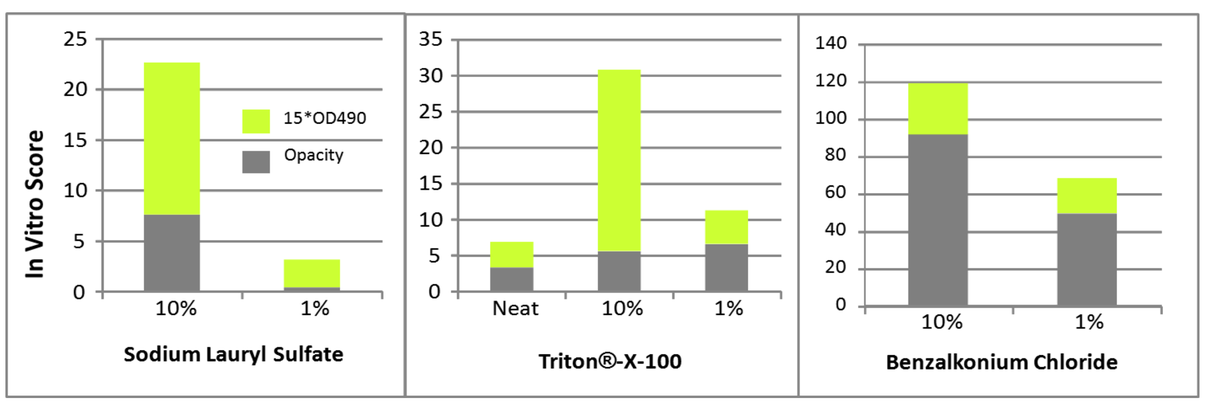


Figure 2. Fluorescein Permeability values (OD_{490nm}) of SLS tested at various exposure times and various concentrations. The results were exposure time-dependent, however, SLS showed an optimal activity at the lower 10% dilution.



In Vitro Score = Opacity + 15 x Fluorescein OD_{490}

Figure 3. Anionic (SLS), cationic (Benzalkonium Chloride) and non-ionic (Triton X-100) surfactants evaluated at various concentrations for an exposure time of 10 minutes. Three corneas evaluated at each treatment condition. Opacity represented by grey bars and OD_{490} represented by bright green bars. SLS and Benzalkonium Chloride were not tested neat since they are solids.
Note the low contribution of opacity to the In Vitro Scores for SLS and Triton. Fluorescein permeability (OD_{490}) is the primary endpoint for resolving among surfactants.

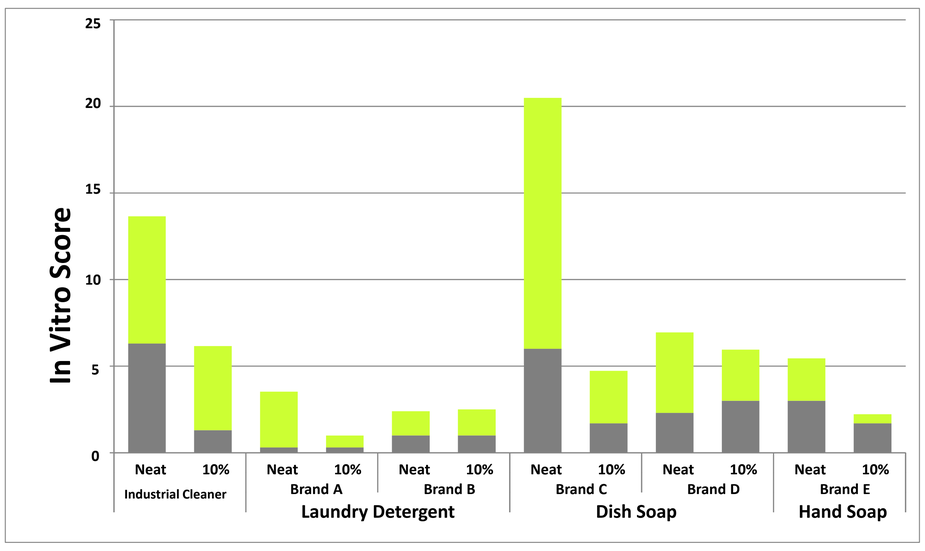


Figure 4. Various surfactant based formulations evaluated neat and at 10% w/v in sterile water for an exposure time of 10 minutes. Three corneas were evaluated at each treatment condition. Opacity represented by grey bars and OD_{490} represented by bright green bars.

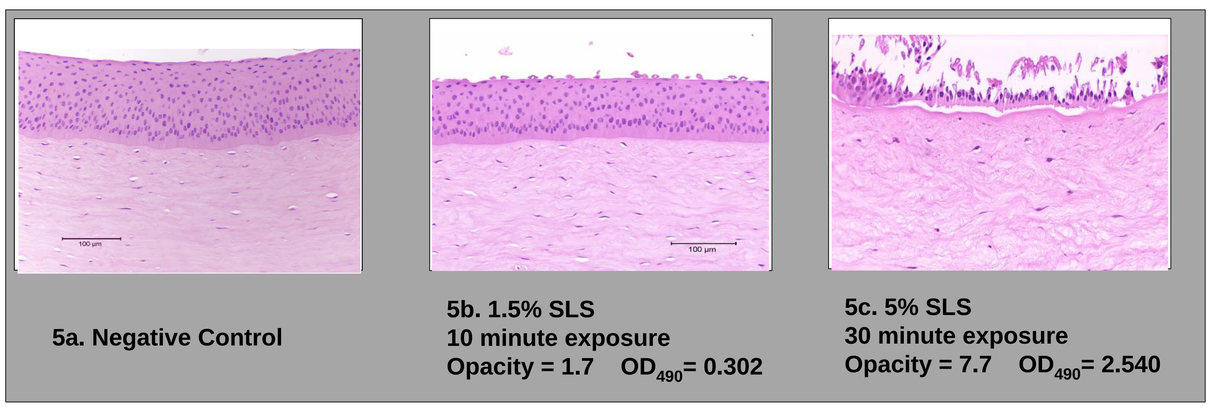


Figure 5. Histopathology Evaluation.
5a. Negative Control cornea showing intact epithelium and organized upper stroma
5b. Loss of squamous and upper wing layers, results in increases in OD_{490}
5c. Complete loss of epithelium, results in high OD_{490}. Marked stromal edema and disorganization results in modest opacity

CONCLUSIONS

- The BCOP assay is well suited to evaluate surfactants and surfactant formulations because it can detect a wide range of irritancy potential (mild, moderate, severe).
- When evaluating surfactants in the BCOP assay, key points (listed in Introduction) should be considered to determine the most appropriate protocols to meet your project goals.
- When evaluating anionic or non-ionic surfactants in the BCOP, the permeability endpoint should be considered independently of the opacity and In Vitro Score, because the opacity may be artificially low (potential for under-prediction).
- Surfactant-induced loss of corneal barrier function is measured objectively by the fluorescein permeability endpoint
- Histological observation supports that the permeability endpoint may be more reflective of corneal damage and therefore a more relevant measurement for eye irritation prediction for certain surfactants than the opacity endpoint.

REFERENCE

[1]Organisation for Economic Co-operation and Development (OECD) Test Guideline: "*Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage*" (TG 437), adopted 26 July 2013.