Protocol Considerations for Testing Surfactants and Surfactant-based Formulations in the Bovine Corneal Opacity and Permeability Assay

Raabe, Hans A., Diersen, Victoria A., Norman, Kimberly G.
Institute for In Vitro Sciences, Inc., Gaithersburg, MD, USA

ABSTRACT

The Bovine Corneal Opacity and Permeability (BCOP) assay is an ex vivo test for predicting ocular irritation. For regulatory classification, OECD Test Guideline (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. Guidance Document (GD) No. 160 also presents that solid and concentrated liquid surfactants may be diluted to 10% for testing. 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. Without question, surfactant solids should not be tested using the solid chemical protocol, since over-exposure conditions are likely. In the absence of clear guidance from these regulatory documents, we present on the testing of a few common surfactant ingredients (sodium lauryl sulfate, Triton X-100, and benzalkonium chloride), and surfactant-based liquid and solid formulations in BCOP using standard and modified dilutions and exposures to evaluate the impact of these variables. Whereas the opacity values for the non-ionic and anionic surfactants were low, changes in the fluorescein permeability values correlated well to expected surfactant activities in all of the surfactant classes tested. Histopathology was performed to confirm corneal changes. We found that surfactants at very high concentrations may not exhibit dose-related effects, as irritation optima may occur at aqueous concentrations between 10 and 30%. Furthermore, since surfactants induce corneal erosion, we advocate that the fluorescein permeability endpoint in the BCOP assay should be evaluated individually from the In Vitro Irritation Score (IVIS) in a hazard assessment. Accordingly, a framework to guide the testing of surfactants and surfactant-based products is presented.

INTRODUCTION

During the development of the BCOP assay, Sina and Gautheron recognized that for some surfactants, typically non-ionics and anionics such as sodium lauryl sulfate (SLS), the In Vitro Irritation Score (IVIS) was under-predicted. The scores obtained from changes in corneal opacity were quite low likely due to the progressive erosion of corneal epithelium by surfactant activity without retention of precipitated corneal proteins associated with corneal opacity. In contrast, cationic surfactants such as benzalkonium chloride (BAK) induced notable increases in opacity presumably from precipitated corneal proteins. It was also recognized that the fluorescein permeability values (FL_{490}) very closely correlated to corneal erosion associated with surfactant activity, but the contribution of the fluorescein permeability value to the IVIS was understated for this class of materials (Cater and Harbell, 2013). Accordingly, various exposures have been proposed which included preparing dilutions of surfactants and surfactant-containing formulations, as well as modifying exposure times from the standard 10-minute exposure to exposures of up to 30 minutes. Whereas surfactant ingredients are generally diluted to 10% in water, the dilution of surfactant-containing formulations has been customized often to fit an exposure scenario. For example, to assess the risk of exposure to shampoo formulations or concentrated liquid hand soaps, dilution to 10% in water models a high concentration to which a consumer may likely be exposed. On the other hand, surfactant cleaners at end-use concentrations (typically found in household spray containers) would not be diluted for testing to reflect the likely exposure to the product contents.

One area of confusion has centered around the testing of solid surfactant-containing products. It should be noted that TG 437 and GD 160 clearly state that solid surfactants may be diluted to 10% and tested according to the liquid protocol; thus, surfactant solids should not be tested using the solid chemical BCOP protocol (i.e. testing a 20% dilution for an inappropriately-long 4-hour exposure). The solid protocol was historically designed as a hazard assessment tool under a worst-case exposure to address aqueous-insoluble pharmaceutical intermediates in an industrial hygiene setting. The testing of surfactants under this protocol would not be relevant, and could be over-predictive. To illustrate the impact of erroneously using the non-surfactant solid chemical BCOP protocol, we purchased several commercially-available surfactant-containing solid products from local retailers and tested them under two conditions; *a)* the TG 437-recommended protocol for surfactants, and *b)* the inappropriate use of the BCOP protocol for non-surfactant solid chemicals (Table 1.).

KEY CONSIDERATIONS FOR TESTING SURFACTANTS:

- Is the surfactant or product testing for regulatory purposes?
  If so, what is the appropriate regulatory protocol per OECD TG 437?
- Is the assay being conducted to support product development? Resolution among prototypes and benchmarks may be enhanced using alternate protocols.
- What are the physiochemical properties of the sample?
  (liquid / solid, single surfactant or mixture, viscosity, charge, pH, other actives)
- Is the sample an ingredient or product formulation?
- What exposure conditions are being modeled (industrial hygiene, transport, end use)?
- Is the sample for professional or home use?
- Is the product a concentrate requiring dilution, or prepared as final formulation?
- If the sample is a formulation, what other components or ingredients may contribute to irritation potential?
- The permeability value generated by the BCOP assay may be the most relevant endpoint for anionic surfactants since opacity (and consequently IVIS) may be low.

MATERIALS & METHODS

Various surfactants and surfactant products were tested in the BCOP assay. The BCOP assay was performed with slight modifications of the methods reported by Sina, *et al*. 1995 (**Fig. 1**). To illustrate the impact of erroneously using the solid chemical BCOP protocol, several surfactant-containing solid-phase products were also tested by inappropriately testing a 20% dilution for a lengthy 4-hour exposure.

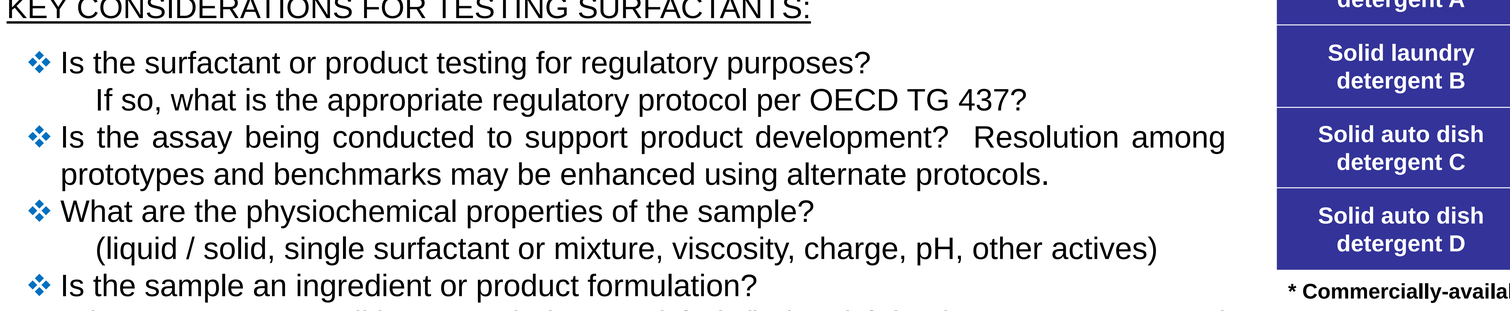


Figure 1. Standard BCOP Assay Procedures for testing liquids – 10-minute exposures

RESULTS

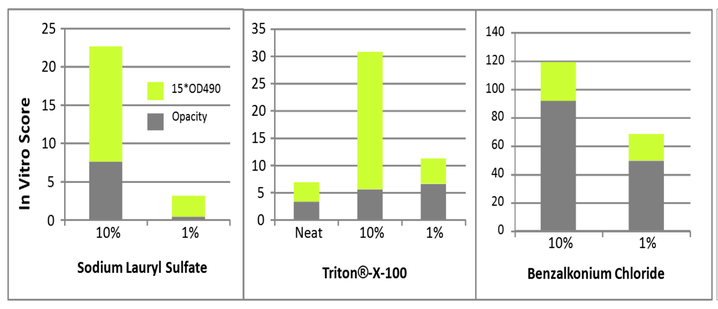


Figure 2. Anionic (SLS), non-ionic (Triton X-100) and cationic (BAK) surfactants evaluated at various concentrations tested at an exposure time of 10 minutes.

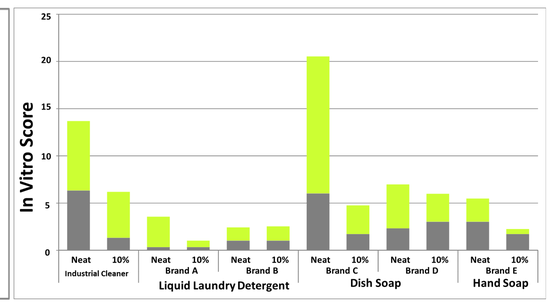
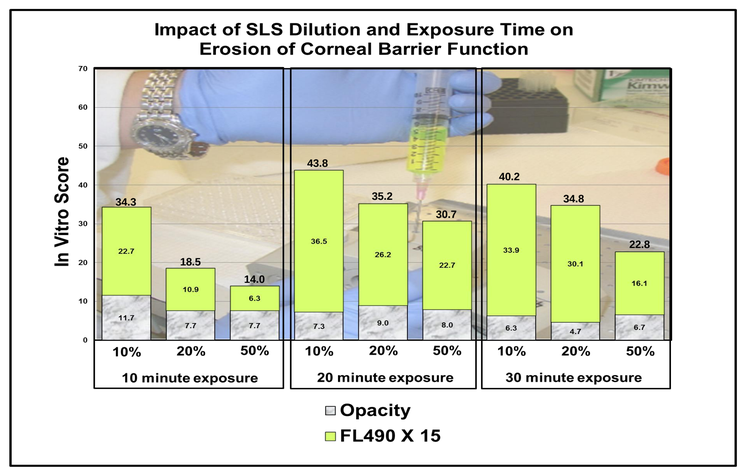


Figure 3. Various surfactant-containing liquid formulations evaluated neat and at 10% w/v in sterile water for an exposure time of 10 minutes.



Opacity values are presented as grey bars and the relative contribution of fluorescein permeability scores ($15xFL_{490}$) to the total IVIS are presented by bright green bars. SLS and BAK were not tested "neat" since they are solids.

Figure 4. SLS tested at various exposure times and various concentrations. Increases in fluorescein permeability (FL_{490}) were exposure time dependent. Importantly, SLS showed an optimal concentration activity at the lower 10% dilution.

Figure 5. Histopathology of progressive surfactant-induced corneal epithelial erosion and stromal swelling.

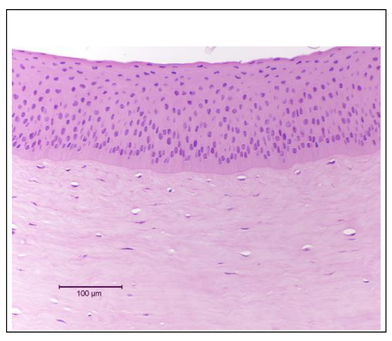


Fig 5a. Negative Control cornea showing intact epithelium and organized upper stroma.

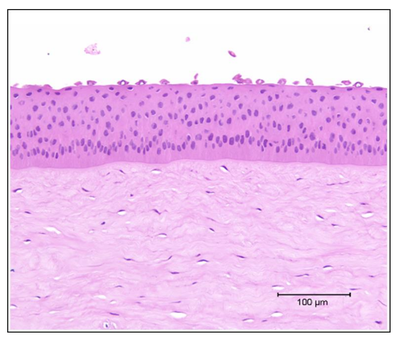


Fig 5b. Loss of squamous and upper wing layers, results in increases in FL_{490}.

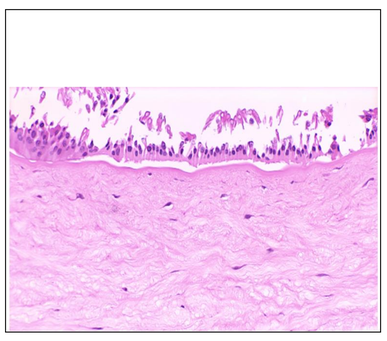


Fig 5c. Complete loss of epithelium results in high FL_{490}. Marked stromal edema and disorganization results in modest opacity.

Table 1: BCOP Test Results

Comparison of Surfactant Solids Test Protocol vs. Non-surfactant Solids Test Protocol

Product Class*	TG 437-recommended protocol for testing surfactant solids (10% dilution for 10 minutes)				Inappropriate use of the non-surfactant solids protocol for surfactant solids (20% dilution for 4 hours)			
	Opacity Value	FL_{490} Value	15 x FL_{490} Value	IVIS	Opacity Value	FL_{490} Value	15 x FL_{490} Value	IVIS
Solid laundry detergent A	0.7	0.048	0.7	1.4	41.7	3.836	57.5	99.2
Solid laundry detergent B	-0.3	0.095	1.4	1.1	23.3	3.765	56.5	79.8
Solid auto dish detergent C	2.0	0.010	0.2	2.2	19.7	1.065	16.0	35.7
Solid auto dish detergent D	0.7	0.010	0.2	0.9	18.7	3.582	53.7	72.4

*** Commercially-available solid laundry and dish detergent products were purchased from local retailers for testing**

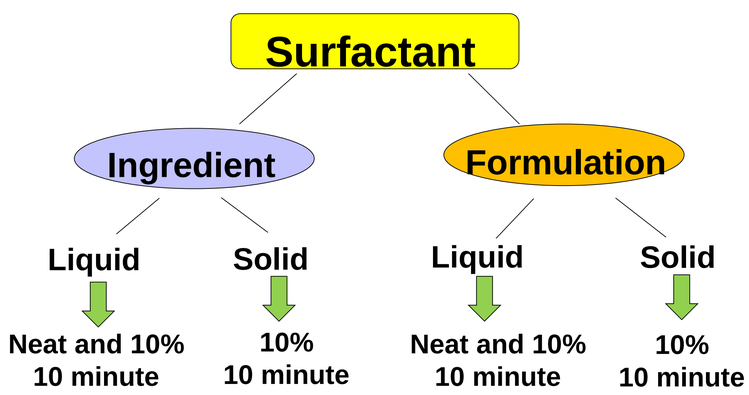


Figure 6. Decision tree for BCOP testing approach for surfactants. For evaluating results from the neat and a 10% dilution, the highest resulting IVIS should be regarded.

CONCLUSIONS

- The BCOP assay can discriminate among a wide range of surfactants with a wide range of irritancy potentials (mild, moderate, severe).
- Inclusion of benchmark materials to interpret test formulation responses enhance product development goals and safety evaluations.
- When evaluating surfactants in the BCOP assay, key points should be considered (see Introduction) to determine the most appropriate protocols to meet project goals.
- When evaluating BCOP results of non-ionic and anionic surfactants, the permeability endpoint should be considered independently of the opacity and IVIS.
- The permeability endpoint is supported by histological observation of corneal epithelial barrier erosion and irritation potential.
- Solid surfactant-containing formulations should not be tested using the standard solid chemical test protocol.

ACKNOWLEDGEMENTS

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REFERENCES

A reprint of this poster and the complete list of references can be obtained at www.iivs.org or by scanning the QR code: