ABSTRACT

The Bovine Corneal Opacity and Permeability (BCOP) assay is an ex vivo test for predicting ocular irritancy. For regulatory classification, OECD Test Guideline (TG) 437 specifies that liquid and solid surfactants may be tested as 10% aqueous dilutions for 15 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 states that surfactant-based formulations are usually conducted 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 fluorescent permeability values correlated well to other endpoints in all of the various studies tested. Histopathology was performed to confirm corneal changes. We found that surfactants at high exposure times 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 fluorescent permeability endpoint in the BCOP assay should be evaluated individually and not be in the Vitro Irritation Score (VIS) 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 (VIS) was under-predicted. The scores changed from changes in corneal opacity were quite low likely due to the progressive erosion of corneal epithylum by surfactant activity without retention of precipitated corneal proteins associated with corneal opacity. In contrast, cationic surfactants such as benzalkonium chloride (BAC) induced notable increases in opacity presumably from precipitated corneal proteins. It was also recognized that the fluorescent permeability values (FLPMD) very closely correlated to corneal erosion associated with surfactant activity, but the contribution of the fluorescent permeability values to the VIS was underestimated 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 the preservative 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 shaft may be expected. On the other hand, surfactant drains 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 risk assessment tool under worst-case exposure to address aqueous-insoluble pharmaceutical intermediates in an intermediate hygiene setting. The testing of surfactants under this protocol would not be relevant, and could be over-predictive. To illustrate the impact of diluting only 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?
- Is the assay being conducted to support product development? Resolution among prototypes and benchmarks may be enhanced using alternate protocols.
- What are the physicochemical properties of the sample?
- Is it solid, liquid, semi-solid, in a suspension, solubility, charge, pH, other actives?
- Is the sample an ingredient or product formulation?
- What exposure conditions are being modeled (industrial hygiene, transport, end-use, etc.)
- Is the product 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 independently testing a 20% dilution for a 4-column 6-hour exposure.

RESULTS

Figure 2. Anionic (SLS), non-ionic (Triton X-100) and cationic (BAC) 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% v/v in sterile water for an exposure time of 10 minutes.

Figure 4. SLS tested at various exposure times and various concentrations, increases in fluorescent permeability (FLPMD) was exposure time dependent. Importantly, SLS and BAC were not tested “neat” since they are solids.

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

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.

Table 1: BCOP Test Results


<table>
<thead>
<tr>
<th>Product Class</th>
<th>TG 437-recommended protocol for testing surfactant solids (10% v/v for 10 minutes)</th>
<th>% Dilution for 10 minute exposure</th>
<th>FLMD</th>
<th>FPM</th>
<th>FLMD</th>
<th>FPM</th>
<th>IS</th>
<th>FLMD</th>
<th>FPM</th>
<th>IS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solid detergent A</td>
<td>0.7 ± 0.046</td>
<td>0.7</td>
<td>1.4</td>
<td>41.7</td>
<td>5.83</td>
<td>57.5</td>
<td>59.2</td>
<td></td>
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</tr>
<tr>
<td>Solid detergent B</td>
<td>-0.3 ± 0.095</td>
<td>1.4</td>
<td>1.1</td>
<td>23.3</td>
<td>3.76</td>
<td>59.5</td>
<td>79.8</td>
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<tr>
<td>Solid detergent C</td>
<td>2.0 ± 0.065</td>
<td>6.2</td>
<td>2.2</td>
<td>19.7</td>
<td>1.00</td>
<td>16.0</td>
<td>35.7</td>
<td></td>
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</tr>
</tbody>
</table>

- Commercially-available solid detergent formulation and diluent were purchased from local retailers for testing

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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.

REFERENCES

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ACKNOWLEDGEMENTS

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