



Evaluation of TRPV1 Activity to Assess the Eye Stinging Potential of Cosmetic Formulations

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ABSTRACT

The Transient Receptor Potential Vanilloid type 1 (TRPV1) receptor is one of the most well characterized pain-inducing receptors and has been recently identified as a valuable tool to predict eye stinging potential of surfactant based formulations. In this study we sought to predict eye stinging of non-surfactant based cosmetic formulations by studying TRPV1 activity using the NociOcular assay. In the NociOcular assay, TRPV1 expressing neuroblastoma cells are exposed to test substance and TRPV1 activity is measured by acute increases in intracellular calcium. Three of the formulations induced stinging in the human test and were also positive in the NociOcular assay. The other four formulations evaluated were classified as stinging in the human test, but a conclusive determination could not be made in the NociOcular assay as the formulations were not fully soluble in assay buffers. The formulations were also evaluated in the EpiOcular™ assay, an established *in vitro* model for eye irritation utilized by the cosmetics industry. The Epiocular™ assay results did not correlate with the human sting data. Our data support that the NociOcular assay may be a valuable *in vitro* tool to predict human eye stinging sensation for cosmetic formulations. Future efforts seeks to further expand the applicability of the assay to product types other than surfactant based formulations.

BACKGROUND

Evaluating ocular irritation is essential to the safety assessment of facial and eye-area cosmetic products. Both *in vitro* and clinical testing methods are extensively used by the personal care industry to evaluate products for eye irritation and eye sensory response. The EpiOcular™ assay is one of the most commonly used *in vitro* assays by the personal care industry because it is a sensitive tissue model which identifies eye irritation potential. The key parameter involved in EpiOcular™ screening assay is the ET₅₀, which is the time required for the product tested to reduce the tissue viability (as measured by the MTT) to 50%, as compared to control treated tissues. The ET₅₀ was chosen because it is an indirect measure of the tissue barrier properties. Eye irritation potential is assessed according to ET₅₀ value; however, no correlation has been established between the ET₅₀ value and sensory response such as eye stinging.

Human clinical studies are a valuable approach to capture both ocular irritation and ocular comfort, including redness, stinging, itching, burning, and eye tearing. However, human clinical testing is only applicable for confirmed mild ingredients or products and limited data is available. In addition to eye irritation, sensory eye response is an important factor for consumer use of personal care products. Therefore, an *in vitro* model capable of identifying the ocular sensory response as a prescreening tool, especially during the formula development phase, would be greatly beneficial.

The Transient Receptor Potential Vanilloid type 1 (TRPV1) receptor is one of the most well characterized pain-inducing receptors. A novel *in vitro* method, NociOcular assay, was recently developed and has been used to predict eye stinging potential for surfactant based formulations. Considering many personal care products are non-surfactant based, an attempt was made to evaluate if NociOcular assay could also be expanded to non-surfactant based formulas for predicting eye stinging potential.

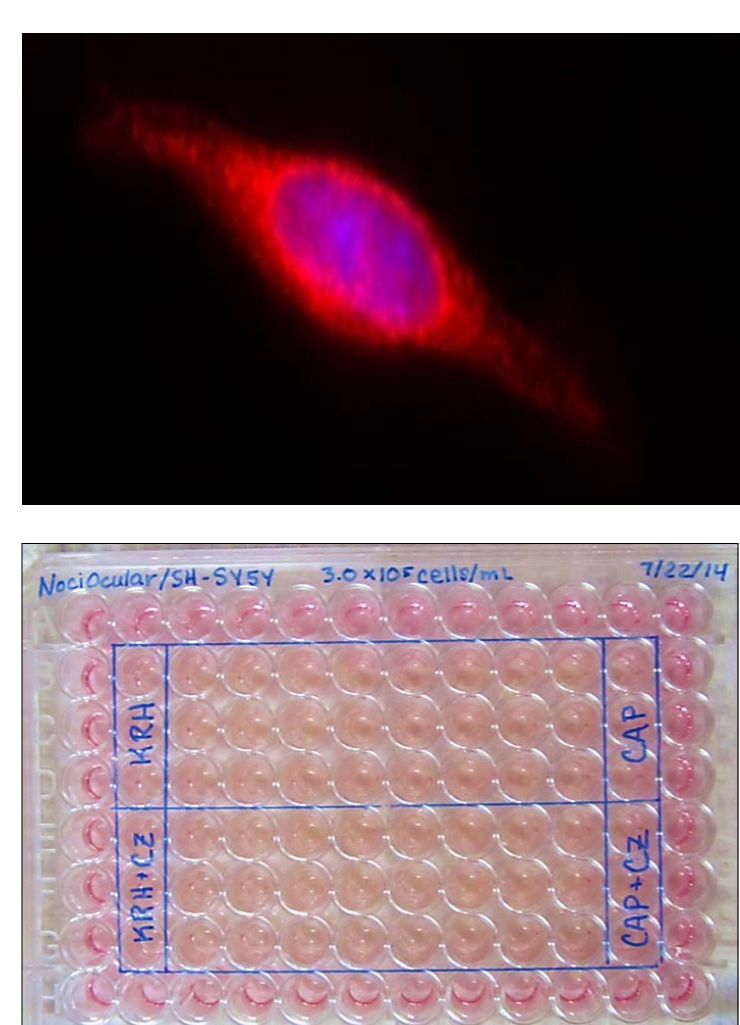
EXPERIMENTAL PLAN

- The NociOcular assay has previously been shown to predict the eye stinging potential of surfactant based formulas. Our goal was to determine if the NociOcular assay could assess eye stinging potential for non-surfactant based formulas.
- Seven prototype personal care formulas with varying degrees of eye stinging potential were selected for evaluation based on ET₅₀ scores from the EpiOcular™ eye irritation assay and/or clinical ocular testing.
- Since these formulas are non-surfactant based, we sought to establish appropriate solvent(s) for use in the assay and dilution scheme(s) which were more relevant to these formulas.
- EpiOcular™ assay (ET₅₀ scores) and/or clinical results and classification were compared with NociOcular assay results.



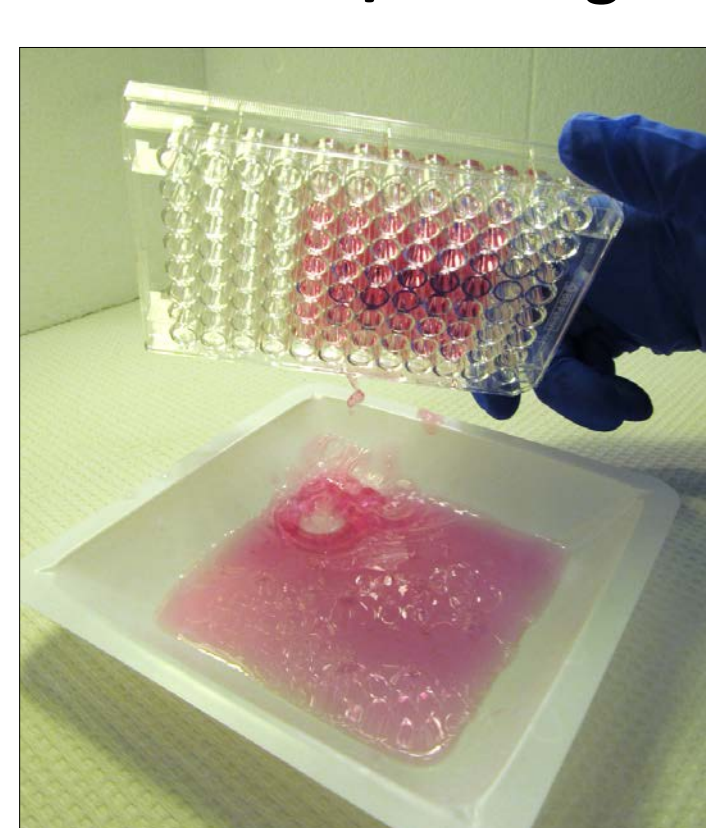
NOCIOCLAR IN VITRO ASSAY

Seeding



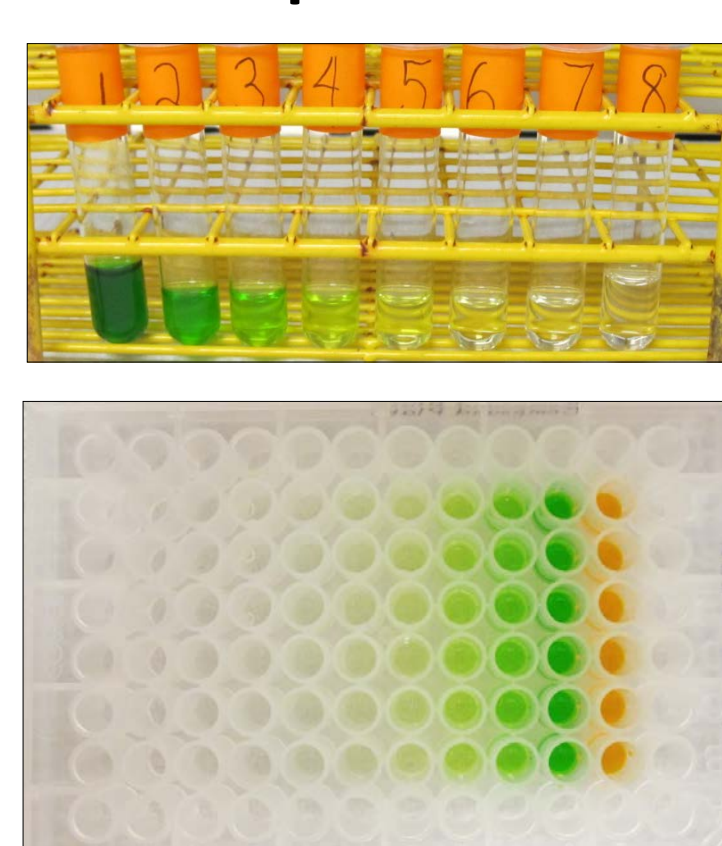
Step 1: TRPV1 transfected SH-SY5Y cells are seeded in 96-well plates and incubated until an appropriate confluency is achieved.

Addition of Calcium Indicator/Rinsing



Step 2: The cells are treated with a calcium dye indicator and rinsed twice prior to the addition of assay buffer. Half of the wells receive buffer with a TRPV1 antagonist (capsazepine).

Test Article Preparation



Step 3: A dilution of the test article is prepared and added to a 96-well compound plate that is later used for dosing. The compound plate also contains the solvent control (assay buffer) and the positive control (TRPV1 agonist) for comparison.

Dose and Read Plate



Step 4: A cell plate, compound plate, and tips are loaded into the FlexStation Fluorometer. The cell plate is systematically dosed and the fluorescence intensity is recorded using SoftMax Pro software. The data is saved and analyzed using SoftMax Pro, Microsoft Excel, and Prism software.

NOCIOCLAR DATA ANALYSIS

- Each formulation was assessed for Ca²⁺ influx over a range of formulation concentrations. The Ca²⁺ influx was compared to the capsaicin response (set to 100%).
- For each formulation an Emax value (% of capsaicin response) was determined.
- To ascertain that the Ca²⁺ influx was due to TRPV1 specific activation, the receptor antagonist, capsazepine, was added to the cells prior to formulation dilutions.
- The seven formulations were ranked according to TRPV1 specific activity using the Emax value and capsazepine responses.

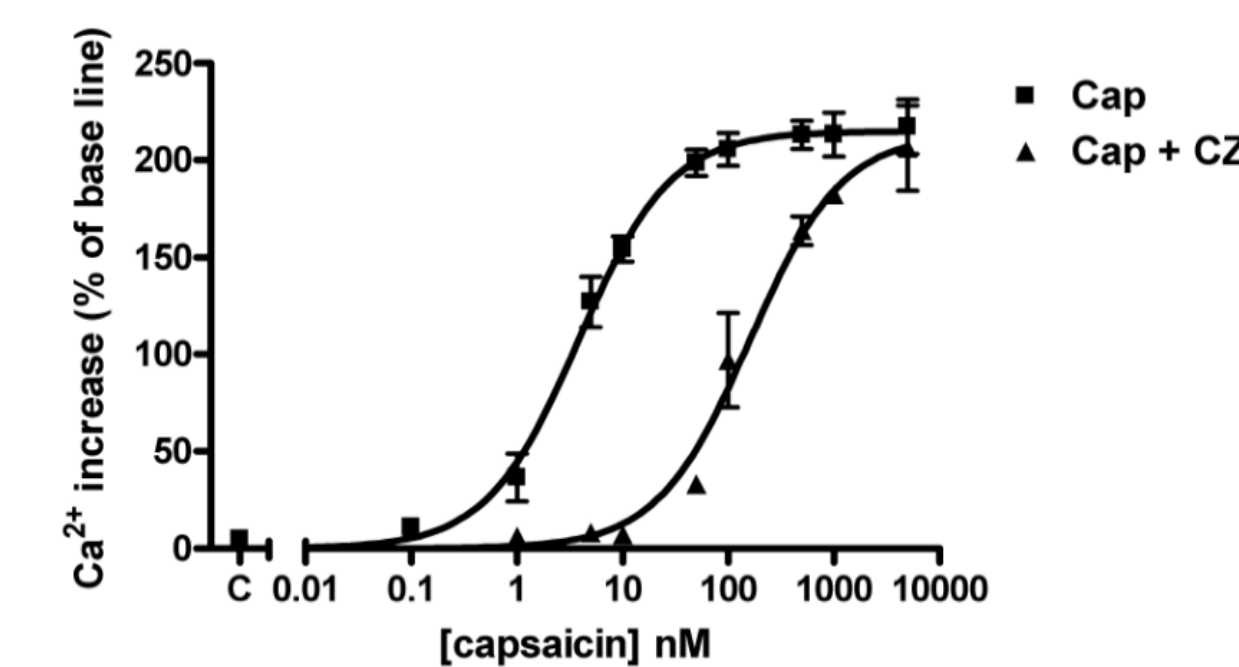


Figure 1. Capsaicin-induced concentration-effect curve of Ca²⁺ in TRPV1-SH-SY5Y cells as measured with the Ca²⁺-binding and fluorescent probe Fura-2/AM. (■) 10 μM capsazepine was added to the wells before capsaicin addition and measurements (▲).

RESULTS

Table 1. Eye Irritation Assessment for each Product using the EpiOcular™ Screening Assay. Each product was evaluated in the EpiOcular™ screening assay and an ET₅₀ value determined. The ET₅₀ value was then used to determine an irritancy rating based on Mary Kay internal assessment scales for each product type. The clinical testing results for eye stinging do not correlate with the ET₅₀ value and irritancy classification.

Product Code	EpiOcular™ ET ₅₀	Eye Irritation Classification	Clinical Testing Results
Facial Mask (Clay)	< 0.5 hour	Irritating	Mild Eye Sting
Facial Mask 01	< 1 hour	Irritating	Eye Sting
Eye Make-up Remover	1.5 hours	Irritating	NA
Facial Mask 02	7.3 hours	Non-irritating, minimal	Mild Eye Sting
Eye Cream 01	> 24 hours	Non-irritating, minimal	Erythema; Eye Sting
Eye Cream 02	> 24 hours	Non-irritating, minimal	Eye Sting
Eye Gel	> 24 hours	Non-irritating, minimal	Eye Sting

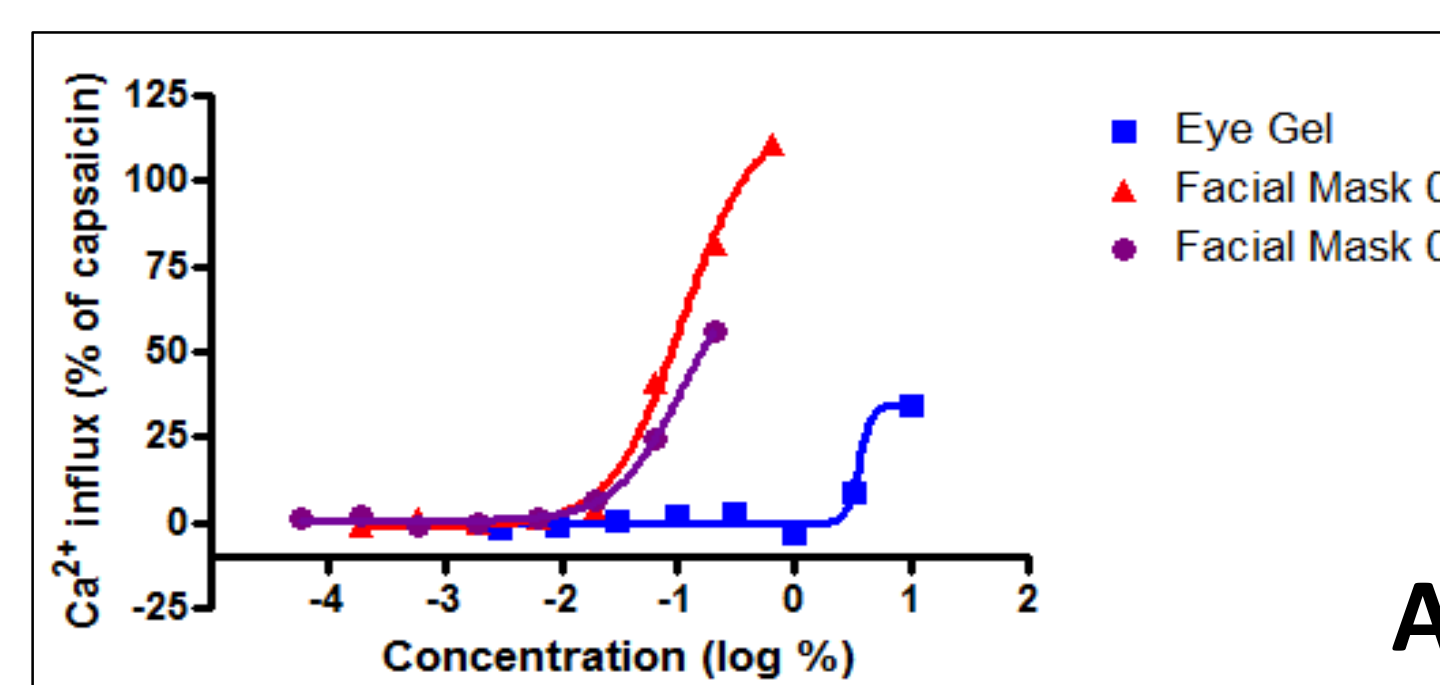


Figure 2. Eye-Stinging Comparison of Formulations Soluble in KRH buffer. KRH buffer is the standard test substance diluent in the NociOcular assay. Only 4/7 formulations were soluble in KRH buffer. The graph shows the concentration effect curves of the 3 formulations which showed TRPV1 specific responses. (A). The range of responses are graded by color ranging from red (strongest TRPV1 response) to blue. The table (B) displays the clinical testing result, determination of TRPV1-specific effect, and Emax values observed for each formulation. Products are ranked according to specific TRPV1 activity, as measured by the Emax value and comparison to treatment with receptor antagonist, capsazepine (not shown).

Product Code	Human Clinical Testing Results	TRPV1 specific response	Emax
Facial Mask 01	Eye Stinger	Yes	118.2
Facial Mask 02	Mild Eye Stinger	Yes	76.8
Eye Gel	Eye Stinger	Yes	34.5
Eye Make-up Remover	NA	No	NA

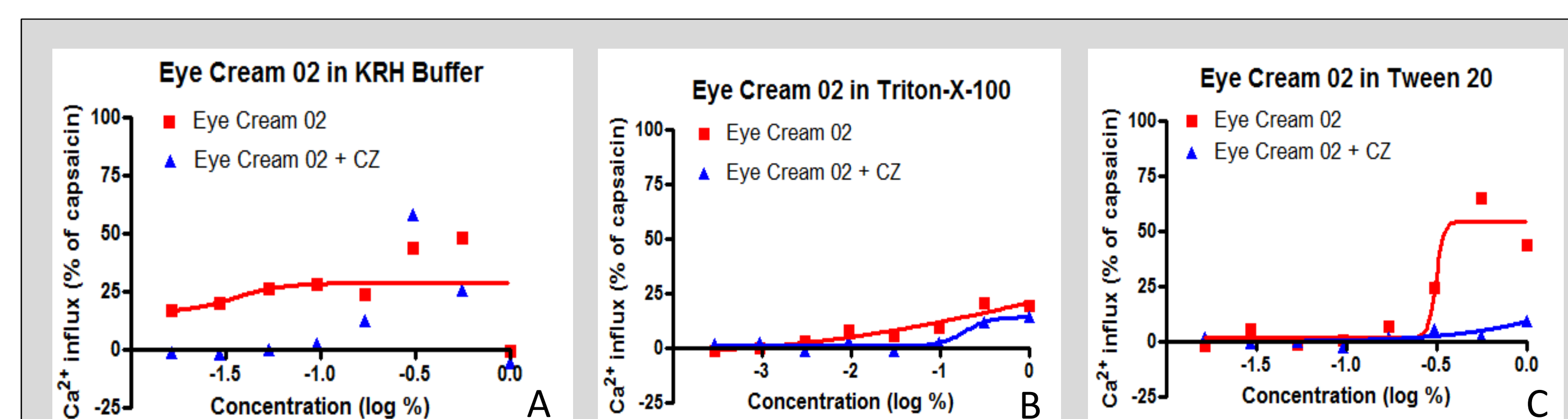


Figure 3. Dose-Response Curves for Eye Cream 02 using different solvents. Each formulation was assessed for solubility prior to the assay. Since KRH buffer is the traditional test substance diluent in the assay, it was first attempted to use KRH buffer as the diluent even if solubility was limited. Eye Cream 02 was first evaluated in the assay using KRH buffer (A) and no dose response was observed. Next, Triton X-100, 0.1% in KRH (B) and Tween 20, 0.2% in KRH (C) were used as solvents. Dilution in 0.2% Tween 20 increased solubility and a TRPV1 specific response was observed.

Table 2. Assessment of Insoluble Formulations. Three formulations were not aqueous soluble and therefore had limited solubility using KRH buffer. Each formulation, except Facial Mask (Clay), was evaluated using KRH buffer, 0.1% Triton X-100, and 0.2% Tween 20 as the formulation diluent. In 0.2% Tween 20 formulation solubility was improved and a TRPV1 specific response was observed for Eye Cream 01 and 02.

Product Type	Clinical Testing Results	NociOcular assay Solvents	Specific TRPV1 response	Emax
Eye Cream 02	Eye Sting	KRH	No	NA
		0.1% Triton X-100	No	NA
		0.2% Tween 20	Yes	54.4
Eye Cream 01	Erythema; Eye Sting	KRH	No	NA
		0.1% Triton X-100	No	NA
		0.2% Tween 20	Yes	44.5
Facial Mask (Clay)	Mild Eye Sting	KRH	No	NA
		0.1% Triton X-100	Not determined; insoluble	NA
		0.2% Tween 20	Not determined; insoluble	NA

CONCLUSIONS & NEXT STEPS

- Of the seven formulations, 4/7 were soluble in KRH buffer (typical buffer used for surfactant based products) and 3/4 were identified as having TRPV1 specific activity which correlated with the clinical results for those 3 formulations.
- Technical challenges with solubility were encountered for 3/7 formulations and alternate solvents including 0.1% Triton X-100 and 0.2% Tween 20 were used to improve solubility.
- Using Tween 20 as solvent improved solubility and a TRPV1 specific response was observed for 2/3 insoluble formulations.
- No solvent was found to be compatible with the Facial Mask (Clay), and further investigation will be conducted.
- Overall, the results of the NociOcular Assay appeared to correlate with the clinical ocular testing for sensorial response and we seek to expand the dataset and further evaluate use of the NociOcular assay as a screening tool for personal care product eye stinging.