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INTRODUCTION

Since the 1980's corion-allantoic membrane (CAM) of embryonic chicken eggs became a model for studying chemicals and products eye irritation potential. HET-CAM was the first test that was developed for this aim and it is criticised due to its low specificity and subjective readings. Trypan Blue staining was proposed in order to be possible to get a quantitative result, but some aspects of the methods must be studied, mainly those related to the behavior of CAM following different surfactants and solvents used in cosmetics and cleaning products.

AIM

This study aims to evaluate the performance of CAM-TBS profile of a dose-response curve of Sodium Dodecyl Sulfate (SDS).

METHOD

Results of *in vivo* Irritation Test (Draize Test) from routine assays using SDS in the INCQS routine were used for comparing CAM-TBS findings. CAM-TBS followed the methodology presented by Hagino *et al.* (1991) and described at INVITOX Protocol number 108. Eleven SDS dilutions were tested (0,03 a 15%) using 10-days incubation Leghorn SPF eggs (4 eggs to each dilution). Test steps are presented at Figure 1. A calibration curve of Trypan Blue was used for calculating the amount of absorbed dye. Irritation grades for each concentration was given depending on the amount of absorbed dye, as described in Box 1.

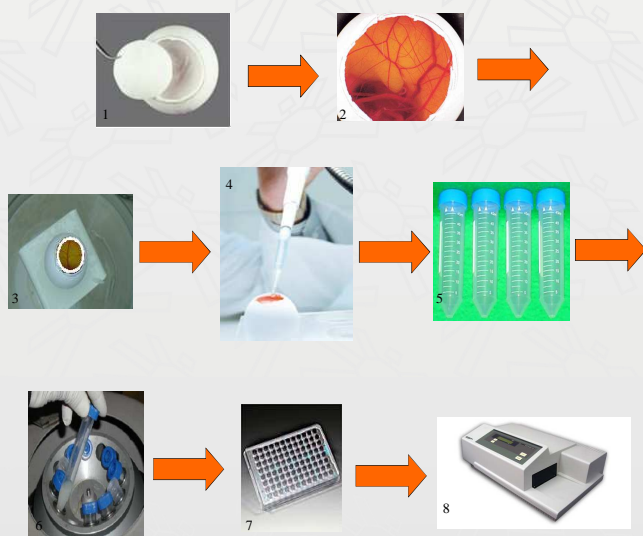


Figure 1: Steps of CAM-TBS assay.

- 1- Removal of eggshell around the air chamber;
- 2- Removal of eggshell membrane and examination of CAM vessels;
- 3- Application of silicon ring on the CAM and instillation of 200 µL of sample;
- 4- After 20 seconds, removal of sample using distilled water;
- 5- Application of 500µl Trypan Blue (0,1%) on the CAM. After 1 minute, removal of dye excess using distilled water;
- 6- Removal of CAM and extraction of dye, in a tube, using 3 mL formamide;
- 7- Centrifugation, 10 minutes, at 3,000 rpm and reading of supernatant (absorption) at 595 nm.

Box1: Classification of irritation potential due to the amount of absorbed dye (Lagarto *et al.* 2006).

AMOUNT OF ABSORBED DYE (nmol)	CLASSIFICATION
< 7,0	Non Irritant (NI) / Mild Irritant (MI)
7,0 – 14,5	Moderate Irritant (Mod)
> 14,5	Severe irritant (SI)

RESULTS AND DISCUSSION

Results obtained from *in vivo* and *in vitro* assays are described in Table 1.

Table 1: Results of Draize test and CAM-TBS for different SDS concentrations.

SDS Concentration(%)	<i>In vivo</i>	Amount of dye (nmol)	Classification	Amount of dye/membrane weight (nmol/g)
0,03	-	0,5	NI	8,1
0,06	-	2,7	NI	33,0
0,12	-	4,5	NI	72,3
0,25	-	4,7	NI	74,6
1	Non Irritant	7,5	Mod	124,9
2	Non Irritant	6,8	NI	81,0
3	Mild Irritant	6,8	NI	66,8
4	Mild Irritant	5,8	NI	57,4
8	Moderate Irritant	5,1	NI	46,4
15	Moderate Irritant	5,8	NI	57,6

Results from *in vivo* SDS assay presented classification of Non Irritant for concentrations of 1 and 2%, Mild Irritant for 3 and 4% and Moderate irritant for 8 and 15%. The concentration x amount of dye curve, for *in vitro* assay presented linearity between 0,03 e 1% concentrations. The Determination Coefficient (R^2) was 0.709 for this part of the curve and it became little higher (0.754) when the amount of dye was expressed in relation to the CAM weight in grams (Figure 2). At concentrations higher that 1% the curve reached its maximum and started presenting descending values. Perhaps it occurred due to a interaction with lipides and other molecules resultins in na epitelial degradation. Another important observation is that the linearity was only observed at non irritant *in vivo* concentrations.

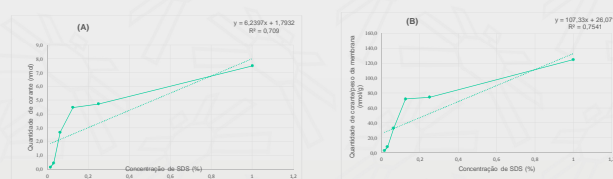


Figure 2: SDS curve for CAM-TBS assay. (A) result expressed as amount of absorbed dye (nmol), em (B) quantidade de corante absorvido por grama de membrana (nmol/g)

CONCLUSIONS

CAM-TBS assay presented presented linearity for diferente SDS concentrations, ranged in those were non irritant for *in vivo* assay. This result suggests that CAM-TBS should be used for determining the absence of irritation potential. Complementary studies are needed to verify the behavior of this assay for other surfactants and to define a predictive model, such as, for example, the establishment of dilution fator for testing fished products.

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