Anti-Pollution Benefits of Sanex Sulfate Free Zero Anti-Pollution Shower Gel

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ABSTRACT

The purpose of this study is to document the benefit of the new Sanex Sulfate Free Zero Anti-pollution shower gel (SG) in more effectively preventing skin lipid peroxidation and removing skin damaging lipid peroxidation byproducts vs. the current Sanex Zero Anti-pollution shower gel on the market. In-vitro pig skin has been successfully used recently to predict anti-pollution skin benefit in-vivo (Bielsfeldt, 2018). Using a novel method we developed recently, pig skin was exposed to ozone to generate lipid peroxides in a well-controlled manner. It was then quantified using a lipid peroxidation (LPO) assay kit. The assay utilizes the colorimetric reaction between thiobarbituric acid (TBA) and malondialdehyde (MDA) to measure the degree of oxidation. MDA is a secondary byproduct of lipid peroxidation and very commonly used as a biomarker for skin lipid oxidation. Results showed that the newly developed Sanex Sulfate Free Zero Anti-pollution shower gel was more effective in removing/reducing free radical byproduct and preventing skin lipid from oxidation vs. the Sanex Zero Anti-pollution shower gel on the market.

OBJECTIVE

Demonstrate the superior efficacy of the newly developed Sanex Sulfate Free Zero Anti-pollution shower gel in preventing skin lipid peroxidation and removing/reducing lipid peroxidation byproducts vs the Sanex Zero Anti-pollution shower gel on the market.

MATERIALS AND METHODS

A CH-1 ozone chamber (Model 106-L, Oxidation Technologies, LLC.) was used for ozone generation.

Pig skin was from Animals Technology, Inc. (Bozman, MD) as a slaughter house waste. Upon receipt, pig skin was immediately frozen and stored at -80ºC.

Lipid peroxidation (MDA) assay kit was purchased from Sigma-Aldrich.

Scrub cups for extraction (inner diameter about 3cm) and glass rods.

Test samples

1. Sanex Zero% Anti-pollution SG
   PDM: 100000175670/000/000
   Lab Notebook: Amira Khan - 00805B

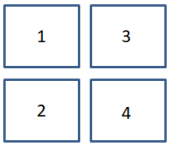
2. Sanex Zero% Anti-pollution Sulfate Free SG
   PDM:100000182231/000/000
   Lab Notebook: Amira Khan - 00805H

Detailed experimental procedures are listed below:

1. Sample preparation:

Prepare samples for MDA removal test

a. Cut two pieces of defrosted pig skin into four 2 inches x2 inches square pieces (as shown below, 1 and 2 are cut from the same piece of pig skin while 3 and 4 are from the same piece). The pig skins marked as 1 and 4 were used for the test product while 2 and 3 were used for control formula respectively.



b. The pig skins were put on top of a weighing paper (VWR 12578-201) and placed in the middle of the ozone chamber, then exposed to 100 ppb ozone for 1 hour for MDA generation.
c. The pig skins were washed in the order of 1, 2, 3, and 4. Tap water was used with 100ml/second flow rate at 100 ± 5 ^{o}F. After pre-wetting pig skin under the running water for 2 to 3 seconds, 200 µl sample or control was lathered on pig skin for 15 seconds. It was allowed to stay in contact with the skin for an additional 60 seconds before rinsing off under tap water for 15 seconds.

Prepare samples for MDA prevention

Same procedures as described above for the removal test were followed but in an altered sequence of a, c, then b instead. Also ozone exposure time was 30 minutes instead of 60 min for the prevention test.

2. MDA extraction: MDA on pig skin was extracted with cup scrub method where 500 µl ethanol was applied twice and each time the pig skin was rubbed with a glass rod for 1 minute. Extracted samples were then centrifuged for 1 minute at 10,000 rpm using Eppendorf centrifuge 5418.

3. Quantify MDA to calculate removal and prevention effect

a. Calibration curve needs to be prepared with each new kit. Malondialdehyde (MDA) was diluted to multiple concentrations from 20 μM of the MDA standard with ethanol, the same solvent used for extraction.
b. Prepare thiobarbituric acid (TBA) solution by mixing one of the four given bottles of TBA powder in the kit, 7.5 ml acetic acid, and 17.5 ml DI water. Mix 600 μl TBA solution and 200 μl samples under test, incubate at 95 ^{O}C for 60 minutes and then cool down the mixtures in an ice box for 10 minutes. Pipette 200 μl of the mixtures into a 96 well plate in duplicates. Measure the intensity of each sample using spectramax M5 (from Molecular Device) - 532 nm of the excitation wavelength and 553 nm of the emission wavelength were utilized for the intensity measurement.
c. Calculate the MDA concentration using the calibration curve.
d. Statistical analysis: The data reported in all tables were analyzed for statistical significance using a two-tailed, two sample equal variance T test.

RESULTS

1. MDA removal

Table 1. MDA levels remained on pig skin after cleaning with shower gels ($p<0.05$)

Sample	[MDA] (μM)		Average	St. dev.
Sanex Zero Anti- Pollution SG (AK-00805B)	3.88	3.71	3.80	0.12
Sanex Sulfate Free Zero Anti-Pollution (AK - 00805H)	2.76	2.30	2.53	0.33

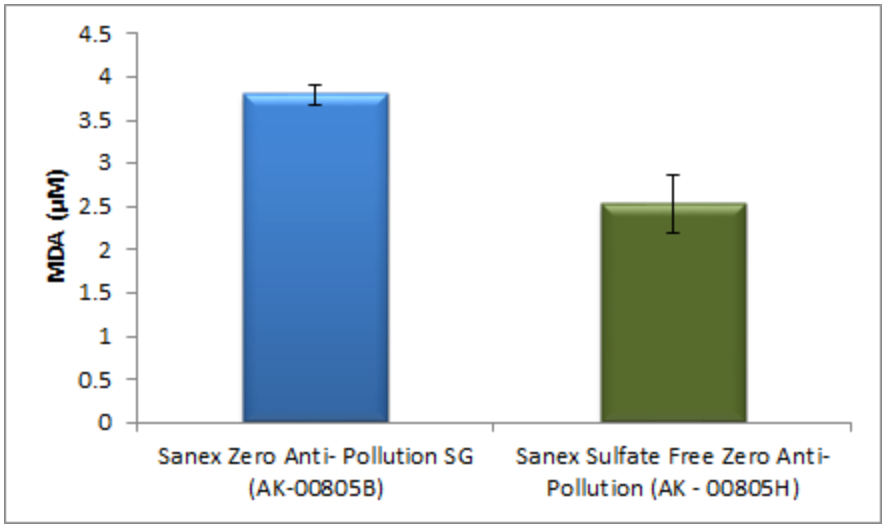


Figure 1. MDA removal/reduction effect of Sanex Sulfate Free Zero Anti-pollution SG vs Sanex Zero Anti-pollution SG (showing MDA levels left on pig skin after the wash)

The results showed that the new sulfate free shower gel generated less MDA than pig skin washed with the Sanex Zero Anti-pollution shower gel. Therefore, it can be concluded that the new sulfate free shower gel more effectively removed/reduced MDA, a skin damaging lipid peroxide byproduct generated when pollutants attack skin.

2. MDA prevention

Table 2. MDA generated when exposed to ozone after cleaning with shower gels ($p<0.05$)

Sample	[MDA] (µM)		Average	St. dev.
Sanex Zero Anti- Pollution SG (AK-00805B)	6.30	6.00	6.15	0.21
Sanex Sulfate Free Zero Anti-Pollution (AK - 00805H)	3.67	3.11	3.39	0.39

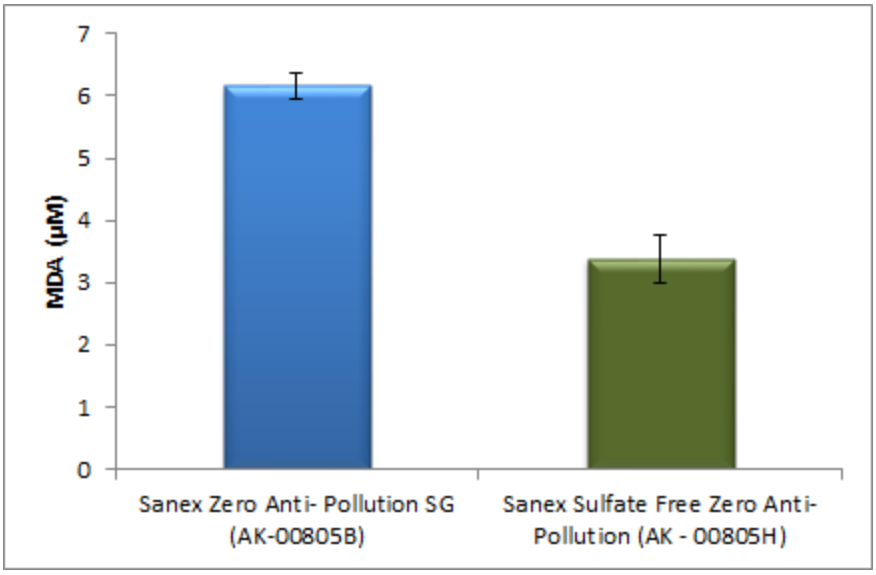


Figure 2. Lipid oxidation prevention effect of the new Sanex Sulfate Free Zero Anti-pollution shower gel vs. Sanex Zero Anti-pollution shower gel showing MDA generated on pig skin when exposed to ozone after washing with the products

The results above showed that pig skin washed with the new sulfate free shower gel generated less MDA vs Sanex Zero Anti-pollution shower gel when exposed to ozone after the wash, therefore providing a stronger prevention benefit.

CONCLUSION

This study demonstrated the anti-pollution benefit of the new Sanex Sulfate Free Zero Anti-pollution shower gel. The demonstrated benefits include more effectively removing skin damaging free radical byproducts and preventing skin lipids from oxidation when exposed to ozone/pollution when compared to the Sanex Zero Anti-pollution shower gel on the market.

REFERENCES

Stephan Bielfeld, etc."Assessment of Anti-Pollution Effects for Two Antioxidants and a Chelator In Vitro and In Vivo on Human Skin by Use of the Cigarette Smoke Model", poster presented at 30th IFSCC Congress (September 18-21, 2018)