

# 2818. Development of an *In Vitro* Whitening Model

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## Introduction

Tooth whitening or bleaching is a popular and established means for removing intrinsic stains. The development of new whitening treatments requires screening of prototype formulations through *in vitro* tests. These tests must be simple, rapid, and predictive of clinical outcome. The treatment is normally applied to the tooth surface for up to an hour and may be repeated twice a day for 7–14 days. Key factors to be considered in the development of an *in vitro* method include both the treatment regimen and the environment. The oral cavity is a dynamic environment where saliva is constantly flowing over the tooth surface. This provides a significant challenge to treatment in terms of substantivity, and it is important that an appropriate challenge is incorporated into the *in vitro* method.

Two studies are reported here using an experimental tooth whitening system containing different levels of hydrogen peroxide (HP) and Crest Whitestrips Classic (CWSC, 6% w/w HP).

## Objective

To develop a simple and rapid *in vitro* whitening model for evaluating the bleaching performance of an experimental tooth whitening system containing HP.

## Methods

Whole bovine teeth were stained in freshly brewed black tea and then mounted in resin such that the facial surface was exposed. The teeth were prebrushed with Macleans Milk Teeth to remove loose extrinsic stain. A baseline L\*a\*b\* was taken for each tooth using a Hunter LabScan XE spectrophotometer. The teeth were randomized, with respect to L\*, and allocated to test groups (n = 7).

The teeth were hydrated in phosphate buffered saline (PBS) prior to treatment. Treatments were applied, as per the manufacturer's instructions, to the tooth surface and held loosely in place with a plastic ring. The treated teeth were placed back in PBS and left for the required treatment time. The treatment was removed by brushing, with a standard toothbrush, under running water, and the teeth were rehydrated between treatments in PBS. L\*a\*b\* readings were taken after the equivalent of 1, 3, and 5 days' treatment. This procedure is shown schematically in the **Process Flow**.

The change in L\*a\*b\* was calculated. One-way analysis of variance (95% LSD) was carried out using StatGraphics Plus software.



PBS, phosphate buffered saline.

## Results

- The results are presented in **Tables 1 and 2** and graphically in **Figures 1 and 2**.
- Both studies demonstrated a dose–response for the experimental tooth whitening system. Despite the large standard deviations, due to the use of a natural substrate, there were statistically significant differences between treatments within individual studies at the 95% confidence level. In study 1 there was a statistically significant difference between the experimental tooth whitening system containing 11.2% w/w HP and CWSC at every time point and between the experimental system containing 6.0% w/w HP and CWSC at Day 1 and Day 3. In study 2 there was a statistically significant difference between the experimental tooth-whitening system containing 5.9% w/w HP and CWSC at every time point.
- By Day 5 the bleaching capacity of the teeth was saturated and discrimination between treatments was reduced.
- The placebo prototype gel shows a response in both studies.

Table 1. Study 1 – Mean (Standard Deviation) Change in L\*

Treatment	Day 1	Day 3	Day 5
11.2% w/w HP	6.00 ± 1.88	9.01 ± 2.82	10.23 ± 3.21
6.0% w/w HP	3.95 ± 1.99	6.50 ± 2.73	7.73 ± 2.92
0% w/w HP	1.57 ± 1.14	2.85 ± 1.54	3.82 ± 1.93
CWSC 6% w/w HP	1.56 ± 0.99	3.66 ± 2.02	5.17 ± 2.81
Water	-0.08 ± 0.26	-0.78 ± 0.47	-0.63 ± 0.55

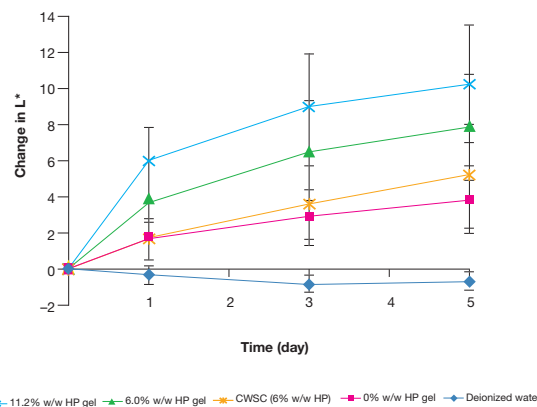


Figure 1. Study 1 – change in L\* with treatment.

Table 2. Study 2 – Mean Change (Standard Deviation) in L\*

Treatment	Day 1	Day 3	Day 5
5.9% w/w HP	5.05 ± 1.94	9.16 ± 2.34	11.12 ± 2.34
3.7% w/w HP	4.05 ± 1.48	7.57 ± 2.54	9.34 ± 2.89
1.1% w/w HP	3.43 ± 1.43	6.72 ± 3.10	8.31 ± 3.90
0% w/w HP	1.71 ± 0.52	2.97 ± 1.14	3.79 ± 1.32
CWSC 6% w/w HP	2.38 ± 0.66	4.72 ± 1.68	6.17 ± 2.07

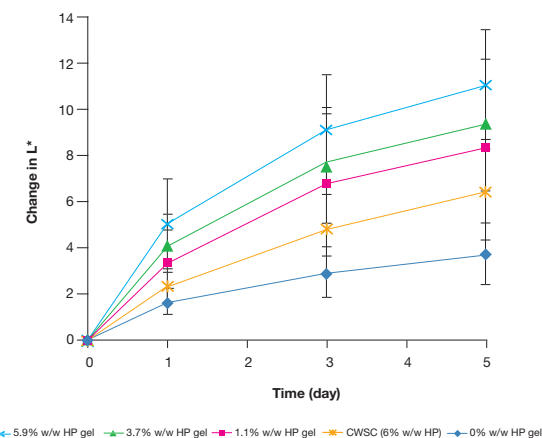


Figure 2. Study 2 – change in L\* with treatment.

## Conclusions

- The *in vitro* model described here provides a good method for evaluating bleaching performance.
- The substantivity challenge incorporated in this model provided a realistic challenge to these treatments.
- The response for the placebo gel may be due to dehydration of the teeth, which is known to have a transient whitening effect.

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