

# 1055. A New *In Vitro* Model for Assessing Dental Stain Prevention

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## Introduction

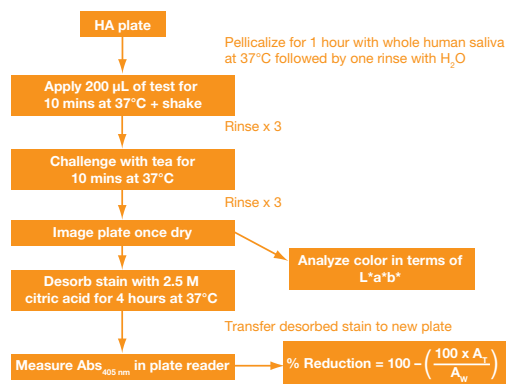
Current *in vitro* models used to investigate dietary staining of teeth either concentrate on measuring the staining potential of an active, such as the antimicrobial chlorhexidine<sup>1</sup>, or model the stain-removal properties of materials such as condensed phosphates<sup>2</sup> and hydrogen peroxide<sup>3</sup>. Published *in vitro* models that do focus on stain prevention use powdered hydroxyapatite<sup>4,5</sup> as a substrate, which is rather difficult to handle and poses problems when trying to quantify stain prevention. *In vivo* methodologies also exist<sup>6,7</sup>, although these models are implicitly unable to split the differing actions of stain removal and stain prevention.

## Objective

To develop a model to rapidly evaluate the potential for a material to prevent subsequent dietary stain and to quantify this effect using spectroscopic measurements.

## Methods

The substrate was hydroxyapatite (HA) powder immobilized on the base of the wells of a 96-well microtiter plate, known as an HA plate. The **Process Flow** below shows the methodology.



Abs, absorbance; A<sub>t</sub>, absorbance from test wells; A<sub>w</sub>, absorbance from water control; HA, hydroxyapatite.

The stain reduction of each test was then calculated using the equation in the Process Flow, where A<sub>t</sub> is the absorbance from the test wells and A<sub>w</sub> the absorbance from the water control. All absorbances are expressed as the average of eight wells and are corrected for solvent absorption. This model is termed the hydroxyapatite assay for stain prevention (HASP) plate.

## Results

- The HASP plate model (Figure 1) was used to demonstrate a dose response for the stain-preventing properties of sodium tripolyphosphate (STP), an active commonly used in toothpastes for its stain removal and anticalculus properties (Figure 2). It is evident that this model is sufficiently sensitive to be able to differentiate the stain prevention between different concentrations of STP.

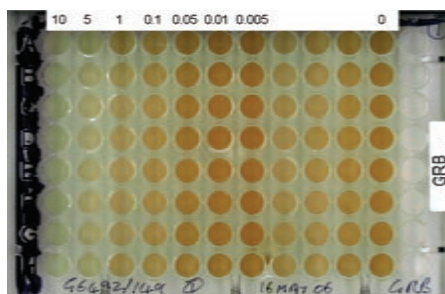


Figure 1. Image of an HASP plate post-tea stain after pretreatment of sodium tripolyphosphate at various concentrations (expressed for each column as % w/w at the top of the image). As a control, the far-right column has not been tea stained.

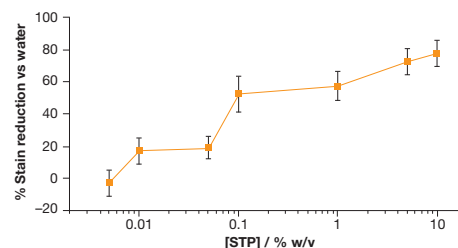


Figure 2. Stain reduction by sodium tripolyphosphate (STP) as a function of concentration. Error bars are % relative standard deviation.

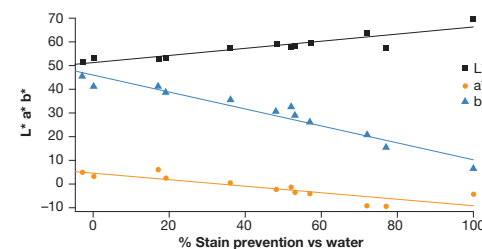


Figure 3. Correlation of stain prevention with L\*a\*b\*. The lines are linear square fits to the data.

- Validation of the desorption process was achieved by correlating the % stain prevention (as measured spectrophotometrically) directly with the color of the wells as measured from the image of the plate. Figure 3 shows that all three of the color axes of the L\*a\*b\* color domain correlate linearly with the stain prevention.

## Conclusions

- The HASP plate model has proven to be a useful and convenient tool for the rapid identification of the stain-preventing properties of toothpaste actives.
- The desorption of stain with citric acid, followed by spectroscopic evaluation of the stain, is by far a more robust means of measuring the stain compared with image analysis, and further does not suffer from image acquisition artifacts such as inter-scanner variabilities.
- The HASP plate model has also demonstrated that STP may be useful as an active in toothpastes to help prevent dental staining as well as to remove existing stains.

## References

- Addy M, Moran J, 1985. Extrinsic tooth discoloration by metals and chlorhexidine. II. Clinical staining produced by chlorhexidine, iron and tea. *Br Dent J* 159:331-4.
- Pontefract H, Courtney M, Smith S *et al*, 2004. Development of methods to enhance extrinsic tooth discoloration for comparison of toothpastes. 1. Studies *in vitro*. *J Clin Periodontol* 31:1-6.
- Dietschi D, Rossier S, Krejci I, 2006. *In vitro* colorimetric evaluation of the efficacy of various bleaching methods and products. *Quintessence Int* 37:515-26.
- Baig AA, Kozak KM, Cox ER *et al*, 2002. Laboratory studies on the chemical whitening effects of a sodium hexametaphosphate dentifrice. *J Clin Dent* 13:19-24.
- Shellis RP, Addy M, Rees GD, 2005. *In vitro* studies on the effect of sodium tripolyphosphate on the interactions of stain and salivary protein with hydroxyapatite. *J Dent* 33:313-24.
- Ayad F, De Sciscio P, Stewart B *et al*, 2002. The stain prevention efficacy of two tooth whitening dentifrices. *Compend Contin Educ Dent* 23:733-6, 738.
- Claydon NC, Moran J, Bosma ML *et al*, 2004. Clinical study to compare the effectiveness of a test whitening toothpaste with a commercial whitening toothpaste at inhibiting dental stain. *J Clin Periodontol* 31:1088-91.