

ABSTRACT

Cetylpyridinium chloride (CPC) is a quaternary ammonium compound, cationic in nature, and has been shown to possess antimicrobial activity against a number of oral bacteria (Smith et al, *J Perio Res* 26:422-428, 1991). Mouthrinses containing CPC have been shown to be effective in the control of dental plaque (Renton-Harper et al, *J. Perio.*, 67:486-489, 1996). A limitation to the use of CPC in oral care products is that the biological activity of the compound can be hindered by other commonly used ingredients in dentifrices and rinses (Addy et al, *J Dent Res.* 72:719, 1993). The objective of the present study was to determine, utilizing an in vitro Disk Retention Assay Model (DRA), whether differences in mouthrinse formulations affect the quantity of available CPC. The study evaluated both commercially available and experimental CPC containing mouthrinses. The following rinses were evaluated: 1.)Scope® (SCP: Procter & Gamble Co., 0.045% CPC); 2.)Cepacol® (CEP: J.B. Williams, 0.05% CPC); 3.)Act® (ACT: Johnson & Johnson, 0.05% NaF/0.05% CPC); 4.)CP1 (placebo, 0% CPC); 5-8.)experimental CPC mouthrinses containing 0.025% (CP2), 0.05% (CP3), 0.075% (CP4), and 0.1% (CP5) CPC. In the DRA, a known amount of test solution is placed on a cellulose filter disk for 1 minute and washed with deionized water by vacuum filtration. Following washing, an acid fuschin dye solution was drawn through the filter paper and subsequently washed 3x with deionized water. The filter paper is then vortexed in a vial containing p-toluene sulfonic acid solution until all visible color is removed. The resulting solution is then syringe filtered into a clean vial. The level of available CPC is determined spectrophotometrically at 545 nm. Results are as follows: 1.)SCP: 0.019%**b**; 2.)CEP 0.027%**c**; 3.)ACT 0.002%**a**; 4.)CP1 0%**a**; 5.)CP2 0.018%**b**; 6.)CP3 0.038%**d**; 7.)CP4 0.054%**e**; 8.)CP5 0.077%**f** (f>e>d>c>b>a;t-test p< 0.05). **These results show that excipient variations in oral rinse formulations do affect the availability of CPC. Mouthrinses formulated for enhanced compatibility had significantly more CPC, as measured by DRA, than the commercially available rinses tested.**

INTRODUCTION

Cetylpyridinium chloride (CPC) is a cationic quaternary ammonium compound that has been shown to possess antimicrobial activity, control of dental plaque. When formulated appropriately, CPC is also effective at reducing gingivitis. The use of CPC in oral care products is limited due to its interactions with commonly used dentifrice and rinse product ingredients hindering its biological activity. An in vitro assay, the disk retention assay (DRA), can be utilized to determine if differences in mouthrinse formulations affect the level of available CPC in the rinse.

OBJECTIVE

The purpose of this study was to determine whether differences in mouthrinse formulations affect the quantity of available CPC as determined by the DRA method.

MATERIALS AND METHODS

Products Tested

1. Scope® (SCP) - 0.045% CPC (Procter & Gamble Company)
2. Cepacol® (CEP) - 0.05% CPC (J.B. Williams)
3. Act® (ACT) - 0.05% CPC (Johnson & Johnson)
4. Placebo mouthrinse (CP1) - 0.0% CPC
5. Experimental rinse (CP2) - 0.025% CPC
6. Experimental rinse (CP3) - 0.05% CPC
7. Experimental rinse (CP4) - 0.075% CPC
8. Experimental rinse (CP5) - 0.1% CPC

Experimental Method

General Method Overview

The DRA method measures the amount of "chemically" available CPC in mouthrinse formulations.

Specifically, the amount of CPC that binds to a cellulose fiber filter during filtration. The bound CPC is reacted with an acid fuschin dye, extracted from the filter disk and assessed colorimetrically for the CPC-fuschin dye complex. The higher the absorbance, the greater the amount of chemically available CPC in the mouthrinse.

Treatment Procedure

A cellulose filter disk (Schleicher and Schuell #740-E) is placed into a clean sample vial using tweezers to prevent contamination of the disk by contact with skin tissue. A 60 ul aliquot of the test sample is pipetted onto the disk and allowed to stand for 1 minute. The filter disk is transferred to a standard aspirator/faucet vacuum pulled filtration system. A 15 ml water wash is suction filtered through the treated cellulose disk to remove the non-bound components of the treatment solution. Next, a 5 ml aliquot of a 0.1% acid fuschin dye solution (w/v) is drawn through the treated cellulose disk, followed by three 15 ml water rinses to remove the excess dye. The cellulose disk is then removed from the filtration system and placed into a clean vial containing 4 ml of a 20% p-toluenesulfonic acid solution (w/v). The disk is then vortexed in the vial until all visible signs of the dye are gone from the cellulose disk. The resulting solution is then syringe filtered through a 0.45 um filter into a clean vial. To the filtered solution, 90 ul of 1 N HCl is added. Absorbance readings are then made spectrophotometrically at 545 nm.

Standard Curve

A series of aqueous CPC solutions containing 0.025%, 0.05%, 0.075%, and 0.1% CPC (w/v), were prepared. This series of solutions, as well as a water blank, were run through the above treatment procedure to generate a standard curve. The standards are curve-fit to a linear regression and correlation coefficients, slope, and intercept are calculated. This results in a linear relationship between CPC-dye absorbance values and CPC concentration (r=0.99875).

The absorbance readings of each of the test solutions is compared to the standard curve, and the level of available CPC is determined. The percent available CPC is then calculated based upon the theoretical /nominal amount of CPC formulated into the product.

RESULTS

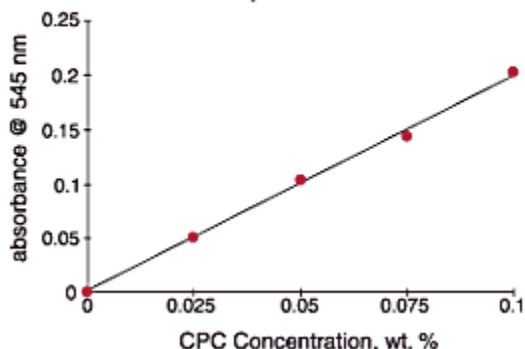
On average, the experimental mouthrinses have between 70-80% available CPC, while Cepacol®, Scope®, and Act® have 54%, 38%, and 4% respectively. Of the 0.05% CPC containing rinses, the experimental 0.05% rinse had substantially more available CPC than Cepacol®, Scope®, and Act®.

Table 1. Available CPC as Determined by DRA

Mouthrinse	Nominal Level CPC (wt%)	Calculated Available CPC (wt%)	Percent Available CPC
SCP	0.045	0.019 (0.004)	41 (7.4)
CEP	0.050	0.027 (0.004)	54 (5.5)
ACT	0.050	0.002 (0.002)	5 (1.8)
CP1	0.000	0.000 (—)	---
CP2	0.025	0.018 (0.001)	71 (6.2)*
CP3	0.050	0.038 (0.005)	75 (6.3)*
CP4	0.075	0.054 (0.009)	70 (8.5)*
CP5	0.100	0.077 (0.009)	75 (5.6)*

() - standard deviation
 * - significant at p < 0.0001 vs commercial products

**Figure 1
DRA Standard Curve
CPC Aqueous Standards**



CONCLUSION

These results show that all formulation excipient variations effect the availability of CPC containing rinses. Formulations optimized for compatibility had significantly more CPC than the commercially available rinses.