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Degradation of Bleaching Gels *In Vivo* as a Function of Tray Design and Carbamide Peroxide Concentration

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Clinical Relevance

Products have been found to degrade at the same rate in trays with or without reservoirs; however, more tray areas without reservoirs had no measurable bleaching agent remaining at the end of two hours.

SUMMARY

This study determined the degradation of nine bleaching agents with different concentrations after two hours *in vivo* following the manufacturers' recommendations. The nine carbamide peroxide products are 10%, 15% and 20% Opalescence, 10%, 15% and 22% Rembrandt and 10%, 16% and 22% Nite White Excel 2.

Each subject wore the tray with the bleaching agent for two hours on three separate occasions. The amount of remaining carbamide peroxide was determined after each use. Evaluation of remaining amount of carbamide peroxide was calculated by the US Pharmacopeia method.

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The study showed that the total carbamide peroxide percent recovered was significantly higher for Opalescence products (47% to 54%) compared to Nite White (22% to 25%) and Rembrandt bleaching gels (15% to 16%). It concluded that this difference was mostly due to the use of facial reservoirs with Opalescence products, and also that whitening gel in trays with reservoirs and trays without reservoirs degraded at the same rate.

INTRODUCTION

One of the reasons for seeking cosmetic dental care is discoloration of the anterior teeth. Even persons whose teeth are a normal color often want them whiter. With careful case selection, diagnosis and treatment planning, bleaching can change a patient's smile dramatically (Haywood, 1997). Bleaching is almost always less invasive and less expensive than other treatment options, such as crowns or veneers, but can be used in conjunction with such procedures when bleaching alone does not completely correct the discoloration or when shape modification is also necessary (Small, 1998).

Although the mechanism by which bleaching agents work is not fully understood, it involves an oxidation process by which the molecules causing the discoloration are chemically modified. The bleaching process

therefore depends on the penetration of the bleaching agent into the source of the discoloration.

The extent to which bleaching works depends on the type of stain, its etiology, the length of time the stain has been present, the bleaching agent itself, how frequently it is applied, how long it remains on the teeth and the concentration that is applied to the teeth (Leonard, Sharma & Haywood, 1998).

It is therefore important to discover how much degradation occurs with different agents. This study determined the degradation of carbamide peroxide (CP) for nine products after two hours *in vivo*.

METHODS AND MATERIALS

Patient Selection

Ten healthy adults were enrolled in this study. Each subject received a dental screening and oral tissue examination prior to starting the study.

Inclusion criteria included: (1) six caries-free, unrestored maxillary anterior teeth; (2) a periodontal disease Gingival Index (Loe & Silness, 1963) <1.0, indicating the absence of periodontal disease; (3) the anatomical crown of the right central incisor being at least 9 mm in length and 8 mm in width; (4) being in a non-smoking state for at least 15 days prior to and during the study and (5) a willingness to sign an informed consent form. The Institutional Review Board of Indiana University-Purdue University Indianapolis approved the protocol and informed consent form.

Nine bleaching agents were used in this study (Table 1).

Procedures

The subjects received a prophylaxis treatment with rubber cup and pumice at least four weeks prior to the study. During the same visit, an alginate impression (Jeltrate Plus, Caulk Division Dentsply International Inc, Milford, DE 19963, USA) of the upper arch of each

subject was taken. The impression was then poured with Super-Die fast setting die stone (Whip Mix Corporation, Louisville, KY 40217, USA) to fabricate a study cast.

Three bleaching trays were made for each subject according to manufacturers' recommendations. Two of the trays had no facial reservoirs (Resin Sheets, Rembrandt Bleaching Gel, Den-Mat Corporation, Santa Maria, CA 93456, USA and EVA Material, Nite White Bleaching Gel, Discus Dental Inc, Los Angeles, CA 90232, USA), and one tray had facial reservoirs (Sof-Tray, Opalescence Tooth Whitening Gels, Ultradent Products Inc, South Jordan, UT 84095, USA).

To fabricate a tray with facial reservoirs, a wax layer of 0.5 mm thickness was applied on the facial surface of the six anterior teeth using 26-gauge casting wax sheets (Kerr Corp, Orange, CA 92867, USA). One millimeter of tooth surface on the mesial, distal and incisal, and 2 mm of tooth surface on the cervical were left without wax. The cast was then immersed in water for five minutes, and a new impression of this cast was taken with alginate and poured to make a second study cast. From the second cast, a customized vacuum-formed stint was fabricated. In all cases, the margins of the tray were trimmed just shy of the gingival margins.

In this crossover design study, three different brands of bleaching gels were tested for degradation of carbamide peroxide after two hours *in vivo*. Each brand had three different concentrations of carbamide peroxide (CP) and each subject tested every product three times using different bleaching trays for each brand. Subjects were provided comfortable chairs and were instructed to refrain from talking, drinking or eating while using the product.

The bleaching agents were analyzed in triplicate before and after the study to determine the carbamide peroxide percentage in the product at both times. A

Table 1: Bleaching Agents Used with Manufacturers, Lot Numbers of Products, Labeled CP%, Assayed CP% Before Beginning of Study and Assayed CP% After Completion of Study

Bleaching Agent/Manufacturer	Lot #	Label CP%	Assayed CP% Before	Assayed CP% After
Nite White Excel 2/Discus Dental Inc, Culver City CA 90232	9FP-9ES	10%	10.886	10.595
Nite White Excel 2/Discus Dental Inc, Culver City CA 90232	9DS-9EA	16%	11.346	10.940
Nite White Excel/Discus Dental Inc, Culver City CA 90232	9GC	22%	22.702	21.683
Opalescence/Ultradent Products Inc, South Jordan UT 84095	37GK	10%	9.713	9.569
Opalescence FP/Ultradent Products Inc, South Jordan UT 84095	3CS7	15%	14.708	14.345
Opalescence FP/Ultradent Products Inc, South Jordan UT 84095	3D8K	20%	19.370	19.001
Rembrandt Xtra-Comfort/Den-Mat Corp, Santa Maria, CA 93456	414996020	10%	10.379	10.239
Rembrandt Xtra-Comfort/Den-Mat Corp, Santa Maria, CA 93456	415996047	15%	17.462	16.819
Rembrandt Xtra-Comfort/Den-Mat Corp, Santa Maria, CA 93456	425996013	22%	25.232	24.585

Label	CP Concentration (%)	Amount of CP (mg)				Recovered Percent
		Delivered	Recovered			
			Tray and Teeth Sample	Rinse Sample	Total	
Nite White 10%	10.9	10.4	1.8	0.5	2.2	23.0
Nite White 16%	10.7	12.1	2.5	0.6	3.1	25.4
Nite White 22%	22.7	28.5	5.4	1.3	6.7	22.4
Opalescence 10%	9.7	10.2	4.9	0.3	5.2	49.0
Opalescence 15%	14.7	19.5	10.1	0.7	10.8	54.1
Opalescence 20%	19.4	21.4	9.8	0.5	10.3	46.9
Rembrandt 10%	10.4	15.2	1.6	0.7	2.3	14.6
Rembrandt 15%	17.5	19.5	2.3	0.9	3.2	15.8
Rembrandt 22%	25.2	33.4	4.1	1.0	5.1	15.3

Bleaching Agent	%CP Tray			%CP Teeth			% Recovered		
	S _b	S _w	ICC	S _b	S _w	ICC	S _b	S _w	ICC
Nite White 10%	1.022	0.448	0.84	2.977	1.095	0.88	18.469	4.605	0.94
Nite White 16%	1.229	0.698	0.76	2.785	1.588	0.75	19.572	8.057	0.86
Nite White 22%	2.544	0.928	0.88	4.102	2.389	0.75	16.006	4.959	0.91
Opalescence 10%	0.463	0.733	0.29	1.166	1.071	0.54	15.135	8.849	0.75
Opalescence 15%	1.151	0.720	0.72	1.709	1.292	0.64	16.342	6.394	0.87
Opalescence 20%	1.833	0.902	0.80	2.559	2.012	0.62	19.484	4.861	0.94
Rembrandt 10%	1.141	0.494	0.84	2.682	2.041	0.63	12.900	5.446	0.85
Rembrandt 15%	2.159	1.117	0.79	4.769	5.354	0.44	13.740	8.857	0.71
Rembrandt 22%	3.264	0.856	0.94	5.159	4.542	0.56	17.864	6.015	0.90

S_b : between subject standard deviation
S_w : within subject standard deviation
ICC: intraclass correlation coefficients

chemistry laboratory technician who was unaware of the specific products being tested. All the bleaching samples for a specific product were from the same lot. Opalescence and Rembrandt bleaching gels were refrigerated during the study as recommended by their manufacturer's instructions. Nite White gels were not refrigerated, as the manufacturer does not recommend it.

The gel was added to a pre-weighed vacuum-formed tray. In the trays without facial reservoirs, the bleaching gel was dispensed in the shape of a line on the facial surface of the tray. For the tray with a reservoir, the gel was dispensed in a zig-zag shape to avoid dispensing excess material into the facial reservoirs. The filled tray was weighed to the nearest milligram on an analytical balance, Mettler AE100 (Mettler Instruments Corp, Hightstown, NJ 08520, USA). The tray was then seated into position in the subject's mouth.

After two hours, the tray with the adherent gel was removed from the mouth and weighed. This was called the "tray sample." It was placed in a beaker with a solution of 100 ml of water and 20 ml of acetic acid. The gel that remained on the teeth was scraped off using a spatula and placed on a tared polystyrene weighing dish and weighed on the analytical balance. This sample was called the "teeth sample." It was placed in a beaker with 100 ml of water and 20 ml of acetic acid. The third sample was called the "rinse sample." It consisted of four gentle mouth rinses using deionized water which the subject expectorated into a beaker after the tray was removed and the gel scraped from the teeth. Twenty ml of acetic acid was added per 100 ml of solution. This sample was not weighed. All samples were analyzed for peroxide content by the method stated in *US Pharmacopeia* (2000).

All the samples were stirred with a stir bar over a stir plate (200-300 rpm) to dissolve the acetic acid into

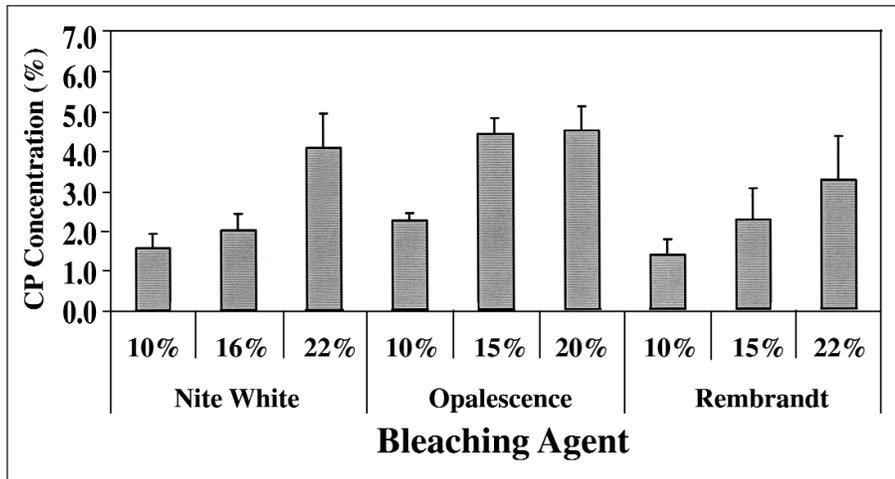


Figure 1. CP concentration in tray and teeth samples combined after two hours (mean and standard error of the mean).

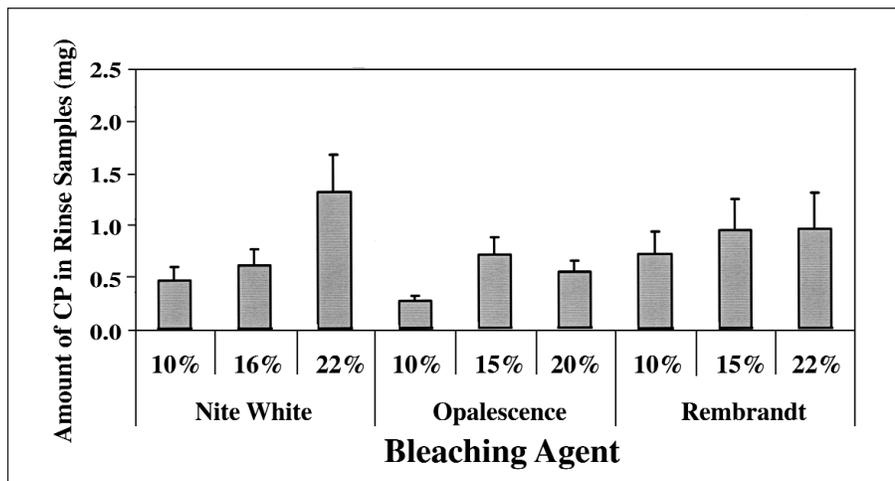


Figure 2. Amount of CP recovered in rinse samples after two hours (mean and standard error of the mean).

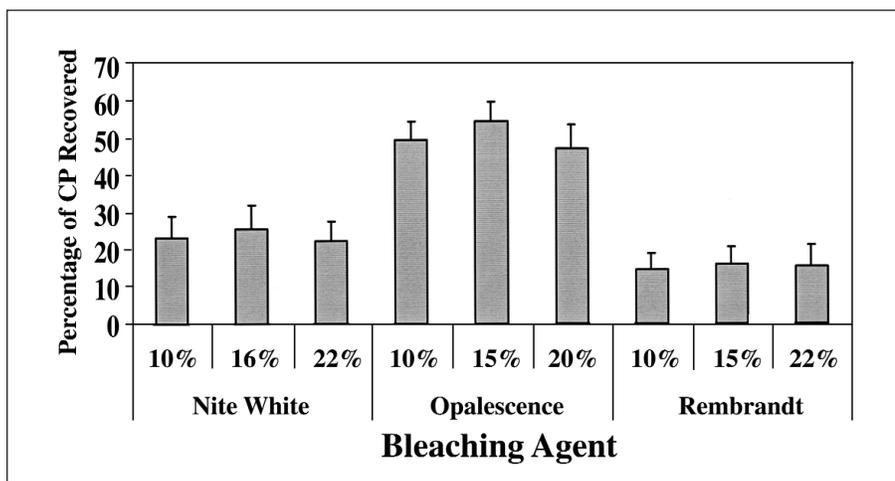


Figure 3. Total percentage of CP recovered after two hours (mean and standard error of the mean).

the solution. After adding acetic acid to all the samples, the beakers were covered using a glass dish, then stirred for approximately five minutes or until the gel was completely dissolved.

Thereafter, 2.0 gm of potassium iodide was added to each sample. The sample colors changed from transparent to yellow depending on the concentration of peroxide in the sample. Two drops of ammonium molybdate were added to each sample by a dropper. The solution changed from yellow to darker yellow or orange depending on the concentration of peroxide in the sample. The glass cover was then replaced on the beakers, and the specimens were placed in a dark area for a minimum of 10 minutes.

Each sample was removed and titrated with 0.025 N sodium thiosulfate. As the titrant was added, the sample turned paler. When the color of the sample was very pale yellow, starch was added. The sample turned purple when the starch was added. The sample then was titrated to the end point, which was reached when the sample color became transparent.

To determine the percentage of carbamide peroxide which was recovered, the following formula was used: $CP\% = V \times 0.025 \times 4.704/W$, where (W) is the total weight of a sample and volume (V) is the amount of thiosulfate used in titration. This formula was applied on tray and teeth samples to obtain the actual concentration of carbamide peroxide. Only the amount of carbamide peroxide was determined in the rinse sample. The result of each sample gave an estimate of the concentration. The percentage of carbamide peroxide recovered was obtained by dividing the total amount of CP recovered by the amount of CP delivered and multiplying by 100.

Statistical Methods

Three replicates per bleaching agent per subject were accomplished. Estimated within and between subject standard deviations and intra-class correlation coefficients (ICCs) were used to represent the repeatability of

the degradation measurements. These values were computed using separate ANOVA models for each bleaching agent, with a random effect for subject. The tray and teeth samples were combined and the average of the three replicates was computed. Comparisons between concentrations and different brands were made using repeated measures ANOVA.

Brands, concentrations and the interaction between brands and concentrations were included in the models as fixed effects. The repeated subject effect was necessary because measurements of each of the bleaching agents made on the same subject may be correlated. Pairwise comparisons between the bleaching agents were made using the Sidak method to control the overall significance level at 5%: adjusted p -value = $1 - (1 - \text{original } p\text{-value})^{\# \text{ comparisons}}$.

RESULTS

Ten subjects participated in this study—eight females and two males. The subjects' mean age was 50 years; they ranged from 27 to 84 years of age. Table 1 shows the percentage of carbamide peroxide for each bleaching gel before initiation and after study completion. After the study was completed, evaluating the bleaching agents showed a slight decrease of values compared to baseline.

CP Concentration in Tray and Teeth Samples

CP concentration in the combined tray and teeth samples was not significantly different for 10% Nite White, 10% Opalescence and 10% Rembrandt ($p=0.70$). CP concentration in the samples for 15% Opalescence was significantly higher than for 16% Nite White ($p=0.0005$) and marginally higher than for 15% Rembrandt ($p=0.08$). However, CP concentration in samples was not significantly different for 16% Nite White and 15% Rembrandt ($p=0.99$). CP concentration in the samples was not significantly different for 22% Nite White, 20% Opalescence and 22% Rembrandt ($p=0.40$) (Table 2, Figure 1).

Amount of CP Recovered in Rinse Samples

Opalescence 20% had a significantly lower amount of CP in the rinse sample than Nite White 22% ($p=0.0125$). Rembrandt 22% did not have significantly different amount of CP in the rinse sample from Nite White 22% ($p=0.78$) or Opalescence 20% ($p=0.71$). The amount of CP in the rinse sample was not significantly different for Nite White, Opalescence and Rembrandt for initial CP percent of 10% ($p=0.58$) or 15%/16% ($p=0.79$) (Table 2, Figure 2).

Total Percentage of CP Recovered

The percentage of CP recovered was significantly higher for Opalescence bleaching gels than for Nite White ($p=0.0003$) and Rembrandt ($p=0.0003$). The percentage

of CP recovered was marginally higher for Nite White than Rembrandt ($p=0.0955$) (Table 2, Figure 3).

DISCUSSION

The methodology used in this study simulates the clinical situation for daytime bleaching. The six anterior teeth were selected for gel retrieval. For this study, subjects with larger teeth were selected to facilitate gel collection from the surface of the teeth. Restoration-free surfaces were chosen to allow for maximum contact of bleaching gel with natural tooth surface. Subjects with no periodontal disease were also chosen to avoid reaction of the bleaching gel with a larger number of microorganisms (Gurgan, Bolay & Alacam, 1996).

Trays were filled but not over or underfilled with bleaching gel. Overfilling would cause physical loss of the material, while underfilling would produce voids that would reduce the reaction of the bleaching gel with the tooth surface.

The Nite White Gel labeled 10% was assayed to have 10.89% carbamide peroxide; the 16% was assayed to have 11.35% carbamide peroxide and Nite White 22% was assayed to have 22.70% carbamide peroxide. Since the 10% and 22% concentrations of Nite White products were within the labeled percentage, the low assay of carbamide peroxide in the product labeled 16% could be due to a quality control problem.

Table 3 shows the repeatability assessment of carbamide peroxide percentage recovered. The repeatability assessment was performed to determine the within-subject consistency of the measurements from three uses of each product. When measurements are inconsistent from multiple uses, measurements should be made multiple times within a subject, then averaged to result in an accurate estimate for each subject. However, when measurements are consistent, multiple measurements from each subject is not necessary to achieve an accurate estimate. Repeatability assessment for tray samples was better than that for teeth samples, however, repeatability was good for all samples.

The concentration of carbamide peroxide in teeth and tray samples, and the amount of carbamide peroxide in rinse samples after two hours *in vivo* were similar among the three brands. For all samples, the higher the concentration of the bleaching gel, the higher the concentration of carbamide peroxide recovered (Figures 1 and 2). This means that using reservoirs did not matter in the rate of degradation and all materials seemed to degrade at the same rate. This shows that teeth should lighten at the same rate regardless of whether or not reservoirs are used as long as there is material present. This would lead the authors to believe that reservoirs are not necessary when bleaching for two hours or less, and other factors besides tooth reactivity with CP, such as heat (temperature of the oral cavity) and saliva, are

involved in the degradation of bleaching agents. However, the total percentage of carbamide peroxide remaining after two hours was significantly higher for Opalescence products (47%-54%) than for Nite White gels (22%-25%) and Rembrandt gels (15% to 16%) (Figure 3). Using facial reservoirs with Opalescence products would explain this.

Trays with facial reservoirs are passive in holding the bleaching gel in the trays and their use limits the amount of bleaching gel that is physically lost during active treatment. The loss in the total recoverable CP is probably due to the dynamic action of the non-reservoir trays trying to reach a resting state.

The degradation results for Opalescence products agree with those found by Gaião (1997). Gaião tested the degradation of 10% Opalescence bleaching gel after one, two, four, six and 10 hours *in vivo*. He found that the percentage of carbamide peroxide remaining after two hours was 52%. The use of facial reservoirs is probably the important factor that resulted in a higher percentage of carbamide peroxide for Opalescence products compared to Rembrandt and Nite White bleaching gels.

In the past, reference has been made to the more rapid degradation of peroxide in proximity to the tooth surface (Matis & others, 1999). This study documents that a minimal amount of peroxide is used in the bleaching process itself, as the concentrations of CP do not differ in the tray and teeth samples. In retrospect, the authors have determined that in the Gaião (1997) study, salivary contamination increased the weight of the sample and, with the amount of peroxide remaining the same, it manifested itself as decreased concentration on the facial and tray surfaces. Wattanapayungkul & others (1999) suspected this concentration gradient error in her study.

Clinical Research Associates (CRA) (1989) found that the percentage of carbamide peroxide remaining after one hour *in vivo* was less than half the original volume. In another study, CRA (1997) found that the percentage of carbamide peroxide remaining after two hours ranged from zero to 30% as the maximum. Ten percent Nite White had 30% of the initial carbamide peroxide remaining after two hours, while 10% Opalescence had 20% of the initial carbamide peroxide remaining after two hours. However, in CRA studies, facial reservoirs were not used. The method of calculating CP was also different from the method used in this study.

The results of the current study could explain the relationship between the percentage of carbamide peroxide and bleaching efficacy. Each manufacturer had 30 trials in each of their three products. In 90 trials of each brand using Rembrandt, no active agent was recovered from seven trials; when using Nite White, no active agent was recovered in nine trials and no active agent

was recovered in one trial using Opalescence. Other studies (Mousa, 1998; Leonard & others, 1998) have shown that the higher the carbamide peroxide percentage in bleaching gels, the faster the bleaching process. In the two-week *in vitro* laboratory study on extracted teeth reported by Leonard & others (1998), a 16% carbamide peroxide solution (Nite White) successfully obtained a three-unit Vita shade change more quickly than 5% or 10% carbamide peroxide solutions. At the end of the two-week regimen of *in vitro* whitening, no significant differences existed in tooth shade among the 10% or 16% carbamide peroxide gel treated groups. However, teeth treated with 10% and 16% carbamide peroxide gel were significantly lighter than those treated with the 5% carbamide peroxide gel. Extending treatment to a third week for the teeth treated with 5% carbamide peroxide gel resulted in lightened shades that approached the two-week values for the 10% and 16% concentrations. The study concluded that lower concentrations of carbamide peroxide gel will whiten teeth and can achieve the same results as higher concentrations; the process just takes longer.

In a clinical *in vivo* study, Hamdan (2002) used 15% Rembrandt bleaching gel in trays with and without reservoirs to evaluate the color change of teeth. Shade guide measurements and a colorimeter device were used to evaluate the color change. He reported that teeth bleached by trays with reservoirs had significantly higher ΔE (total color difference) than teeth bleached by trays without reservoirs. ΔE was 3.36 for the reservoir group, while it was 3.05 for the no reservoir group. This means that teeth bleached by trays with reservoirs were significantly lighter than the other group. However, this difference was not significant for shade guide readings or photographic assessment. Clinically, the difference between both groups would be detectable by the human eye if the difference between ΔE for both groups was one point or higher (Seghi, Hewlett & Kim, 1989; Kuehni & Marcus, 1979; Rubino & others, 1994). These results would indicate that for two hours of daytime home bleaching, the presence of reservoirs would not lead to a clinically significant color change compared to trays without reservoirs.

This study showed that much more bleaching gel is lost into the oral cavity and probably ingested by subjects when reservoirs are not used. In this study, more than 25% of the CP was ingested when the subjects did not use reservoirs. Wattanapayungkul & others (1999) reported that the collected saliva from subjects using bleaching trays with reservoirs revealed an average of 2.10 mg or 6.6% of the carbamide peroxide placed in the tray after one hour of bleaching. This number, however, would increase if the bleaching trays did not have facial reservoirs. Also, this number could decrease if the subject wore the tray at night when the salivary flow and the frequency of swallowing is decreased.

This study has shown conclusively that much more whitening gel is lost to the oral environment when reservoirs are not used. In the past, when a negligible amount of gel was lost, one could refer to it as degradation of the gel; however, now one must begin referring to the kinetics of active ingredient release (American Dental Association, 1998).

The amount of active ingredient recovered needs to be determined whether the breakdown is a chemical breakdown of the agent, a breakdown due to antioxidants on the surface of the teeth or a physical loss of the product. The ADA guidelines for acceptance of a product specifically refer to the necessity of determining the kinetics of the active ingredient release of whitening gels before accepting any product. In the future, one must refer to this physical and chemical loss of active agent as the kinetics of whitening agents, now that whitening agents are being used in trays without reservoirs.

CONCLUSIONS

This study evaluated the degradation of nine bleaching gels after two hours *in vivo*. Ten healthy adults participated in the study over a period of four months. Three different brands of bleaching gels were tested (Nite White, Opalescence and Rembrandt). Each brand had three different concentrations of carbamide peroxide. The manufacturers of Nite White and Rembrandt recommended no reservoirs in trays holding their products, and the manufacturer of Opalescence recommended facial reservoirs in trays holding its products. Each subject tested every product three times using different bleaching trays for each brand. Each subject made a total of 27 visits. After two hours of wearing the trays, the bleaching gel was retrieved from the surfaces of teeth, from the bleaching trays and intraorally by four mouth rinses with deionized water. Analyses of carbamide peroxide were conducted following the US Pharmacopeia method.

The study concluded that:

1. All nine products tested degraded at the same rate.
2. Total recovery of carbamide peroxide was significantly higher when reservoirs were placed in trays.
3. More carbamide peroxide is ingested when used in trays without reservoirs.

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