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# Evaluation of antimicrobial property of modified acrylic resin–containing cetylpyridinium chloride

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## Abstract:

**OBJECTIVE:** To evaluate the antimicrobial property of cetylpyridinium chloride (CPC) when polymerized with cold cure acrylic and to assess the duration of its release from modified acrylic.

**MATERIALS AND METHODS:** CPC was added in different concentrations (0%, 2.5%, 5%, and 10%) to cold cure acrylic resin and 180 acrylic discs were prepared. These were divided into four groups of 45 each depending on the concentration of CPC. The antimicrobial property of the modified acrylic for *Streptococcus mutans* was tested using disc diffusion assay in agar. The duration of release of CPC from self-cure acrylic was tested with optical density reading of solutions by ultraviolet spectrophotometer. The effect of addition of CPC on diametral tensile strength (DTS) of acrylic was tested using UTM (Instron) and the effect of water aging on modified acrylic was compared with unaged specimens.

**RESULTS AND CONCLUSION:** The normality of the data was checked by Shapiro–Wilk test, and as the data failed to show normal distribution, inferential statistics were performed using nonparametric tests of significance. Antimicrobial activity of modified acrylic increased with increase in CPC concentration. Greatest CPC release was observed on the seventh day with a decrease in release from 7 to 180 days. There was a decrease in the diametral strength of the modified resin and water aging had a significant effect on the DTS of the modified resin.

## Keywords:

Cetylpyridinium chloride, modified acrylic resin, *Streptococcus mutans*

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## Introduction

Orthodontic appliances are considered to be a clinical risk factor in terms of enamel integrity because of biofilm accumulation on their surfaces.<sup>[1]</sup> Increased levels of mutans streptococci and lactobacilli have been detected in the oral cavity following orthodontic treatment.<sup>[1-3]</sup> Even after completion of active orthodontic treatment, the majority of orthodontic patients are required to wear retention appliances for prolonged periods of time.

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The efforts to prevent demineralization in these risk groups have been targeted on direct suppression of the cariogenic microflora by chemotherapeutics as an adjunct to oral hygiene, such as fluoride toothpaste, fluoride varnish, and mouth rinses, and numerous formulations are available in the market.

Cetylpyridinium chloride (CPC) monohydrate is used as an antibacterial agent in dentistry. It is used principally as a mouthwash. The quaternary ammonium compound CPC is a cationic surface-active agent and has some similarities to chlorhexidine in this respect.<sup>[4]</sup> CPC is effective because it has a

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broad antimicrobial spectrum with a rapid bactericidal effect on Gram-positive pathogens and a fungicidal effect on yeast in particular.<sup>[5]</sup> It is assumed that interaction with bacteria occurs by causing disturbance of the membrane function, leakage of cytoplasmic material, and ultimately the collapse of the intracellular equilibrium.<sup>[5,6]</sup> Previous studies<sup>[7-9]</sup> have clearly shown that CPC has good antimicrobial property, but due to its low substantivity it does not provide adequate plaque control for a long duration. During the past decade, interest has grown in the use of carriers and slow-release agents for CPC in the mouth.

A slow-release system of the CPC incorporated into cold cure acrylic resin could overcome the problem of its low substantivity and suppress the plaque bacterial growth. The objective of this study was to evaluate the antimicrobial efficacy of a modified self-cure acrylic containing CPC and its duration of release.

## Materials and Methods

Acrylic resin discs incorporating different concentrations of CPC were prepared. A total of 180 discs were prepared and divided into four groups of 45 each, depending on the concentration of CPC. The distribution of groups is shown in Table 1.

### Preparation of acrylic resin discs

Wax models were used to fabricate moulds to prepare acrylic discs of uniform size (4 mm in diameter and 2 mm in thickness). Bioplast sheet (2 mm) was used to prepare the mould from the wax models. For preparing discs of different test groups, the polymer and CPC powder were weighed with an electric digital scale according to the respective concentrations of each group. After mixing, both the powders were kept aside. For differentiation, four colors were used with monomer liquid for each of the group. Colors were mixed with the monomer liquid sufficiently so that it became slightly translucent.

For polymerization, a polymer monomer ratio of 3:1 was taken, as advised by the manufacturer. Monomer liquid was poured in the mould upto one-fourth of its depth and the respective polymer-CPC powder mixture was carefully sprinkled over the monomer till

it filled the mould completely. The polymer powder was allowed to get wet when sprinkled. The excess material was carefully removed. Once it reached the stringy stage, it was allowed to polymerize completely. When polymerization was completed, the discs were separated from the moulds and stored at room temperature. The same procedure was repeated to fabricate all other discs. All the samples were prepared on the same day to prevent any error. These acrylic specimens, containing CPC, were the “modified acrylic discs.”

### Bacterial inhibition

All the polymerized discs were stored in test tubes containing 10 mL of sterile distilled water for 196 days. Three discs from each group were withdrawn for testing after every 14 days. The disc was removed from its test tube, air-dried, and tested for antimicrobial activity using disc diffusion assay in agar. For this assay, BHI agar plates were used. They were inoculated with *Streptococcus mutans* 10449, using a 60-fold diffusion of a 0.5 optical density at 550nm cell suspension prepared from a 24-h culture. The inoculum ( $0.5 \times 10^5$  colony-forming units) was spread aseptically and uniformly on the agar plate surface with a sterile inoculating loop. One agar plate was used for four acrylic discs (one from each test tube); thus, three agar plates were required for each sampling period. Three discs from each test group were placed on the surface of the preinoculated agar plate and incubated for 48 h at 37°C [Figure 1]. Conventional microbiology laboratory procedures were followed and the colony morphology of *S. mutans* on agar plates was examined visually. After 48 h, the growth inhibition zone around each disc was measured and recorded. This procedure was repeated every 14 days upto 196 days [Figure 2].

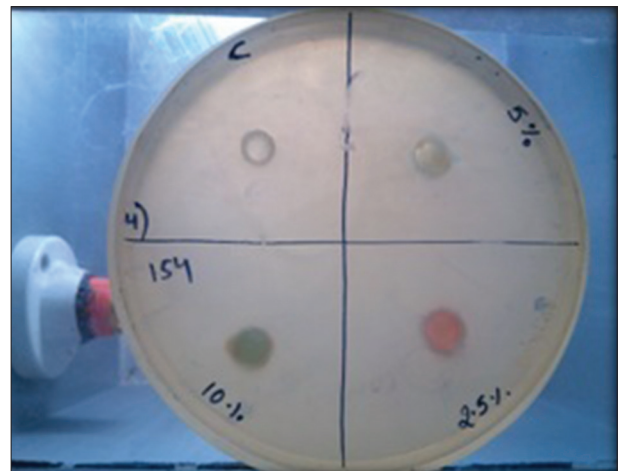
### Amount of release of CPC

The *in vitro* release of CPC from CPC-adhesive discs was evaluated over 180 days with the optical density reading of the solutions at 254 nm using an ultraviolet

**Table 1: Distribution of groups depending on concentration of CPC**

Groups	Concentration of CPC	Sample size	Color used
I (Control)	0%	45	Yellow
II	2.5%	45	Red
III	5%	45	Green
IV	10%	45	Blue

CPC: cetylpyridinium chloride



**Figure 1: Agar plate with disc from each group**

spectrophotometer. Twelve additional CPC-acrylic discs were prepared as previously described, three for each test group. Each disc of each test group was placed in a sealed glass test tube with 3 mL of sterile distilled water. The amount of CPC released into the water from each disc was measured and recorded on days 7, 15, 30, 60, and 180. For this, 200  $\mu$ L of the solution was withdrawn from each tube, and the amount of CPC released from the test disc was determined by optical density at 254 nm; these values were then converted to micrograms per milliliter CPC equivalent with a standard calibration curve. At each sampling time, the disc was removed from the test tube, air-dried, and transferred into another test tube containing 3 mL of fresh distilled water. After 7 additional days (day 15), CPC release was determined as described. Thus, measurements were taken on days 7, 15, 30, and 60, and this process was repeated for each disc in each test tube [Figure 3].

### Diametral tensile strength and water aging

The diametral tensile strength (DTS) of the modified acrylic discs was tested using a Universal Testing Machine (Instron). Additional adhesive discs were prepared as described previously, 20 for each group.

A week later, 10 specimens were tested for each concentration (total of 40 discs). Testing was in compression; specimens were placed centrally on the loading platform with the disc diameter in a vertical position. The specimens were tested for DTS at a crosshead speed of 2 mm/min. The DTS in megapascal for each sample group was determined by the following equation:

$$DTS = (2P/\pi d t) \times 9.8067$$

Where  $P$  is the load in kilograms,  $d$  is the diameter in millimeters,  $t$  is the thickness in millimeters, and 9.8067 is the conversion factor for kilograms to Newton [Figure 4].

The remaining 40 CPC-adhesive discs were placed in distilled water at 37°C for 180 days and then tested to evaluate the effect of water aging on DTS [Figure 5].

## Results

Data were analyzed using SPSS version 21. The normality of the data was checked by Shapiro–Wilk test. Kruskal–Wallis test was used to compare the zone of bacterial inhibition and DTS followed by *post hoc* comparison by Mann–Whitney  $U$ -test. The amount of CPC release was assessed using Friedman's test followed by *post hoc* pair wise comparison by Wilcoxon signed-rank test. The level of significance was set at 0.05.



Figure 2: Diametral tensile strength calculation with Universal Testing Machine



Figure 3: Amount of CPC release checked through spectrophotometer

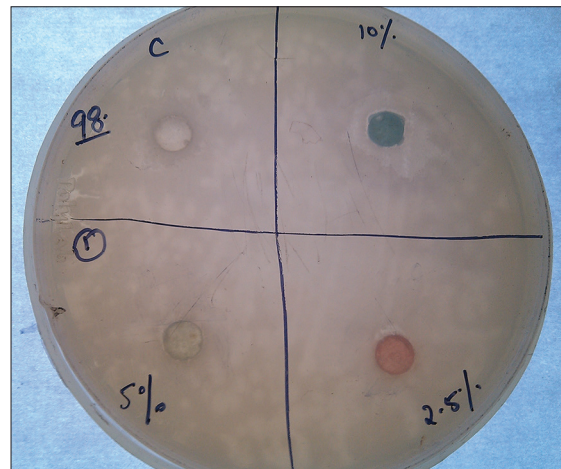


Figure 4: One agar plate used for four acrylic discs (one from each group) to evaluate growth inhibition zone around each disc

### Bacterial growth inhibition with disc diffusion assay

The CPC-acrylic specimens showed significant antimicrobial activity when compared with the controls,



which had no inhibition zone around its specimens when placed on BHI agar plates pre inoculated with *S. mutans*. The antimicrobial activity of the modified acrylic increased with the concentration of CPC. Discs containing 10% CPC had the maximum antimicrobial activity when the zone of inhibition was measured at each sampling time [Table 2]. Upon storage in distilled water, all CPC-containing acrylic discs retained antimicrobial activity over the 196-day testing period. The highest antimicrobial activity was observed on day 1 with large zones of inhibition of 1.5–2.15 mm. As time of aging increased, a decreasing trend was seen in the zones of bacterial inhibition.

**Amount of CPC release**

The amount of CPC released showed a decreasing trend with an increase in time for all the CPC-containing

**Table 2: Bacterial inhibition (in mm) around the acrylic discs in all groups**

Days	Group I	Group II	Group III	Group IV
1	0	1.7	1.5	2.15
14	0	1.475	1.65	1.7625
28	0	1.225	1.4	1.9375
42	0	1.2	1.475	1.675
56	0	1.15	1.2	1.675
70	0	1.3	1.35	1.6125
84	0	1.3	1.65	1.6725
98	0	1.2	0.95	1.2
102	0	1.225	1.325	1.4
116	0	0.975	0.9875	1.175
130	0	1.6125	1.2	1.8
144	0	1.1125	0.975	1.1625
158	0	1.125	1.1625	1.175
172	0	1	1.1125	1.0625
196	0	1.475	1.35	1.15
Mean±SD	0±0.00	1.28±0.00	1.24±0.00	1.51±0.00
P		<0.001*		
Post hoc pairwise comparison		1<2,3,4		

\*P<0.05. SD: Standard deviation

discs. However, only group II and group III showed a significant decrease in CPC release when compared from day 7 to day 180. In group IV, the decrease in CPC release was not significantly different from day 7 to day 180 [Table 3]. Increasing the concentration of CPC resulted in a significant increase in CPC release, from 0.9 ± 0.02 g/mL (2.5%) to 1.99 ± 0.01 g/mL (10.0%) after 7 days of water aging. The greatest CPC release was observed on day 7 for all CPC acrylic discs. The CPC release on days 15, 30, 60, and 180 was significantly lower than those on day 7 in all the groups.

**DTS of dry and wet specimens**

DTS was calculated from the peak load. Acrylic discs with the highest CPC content (10.0%) showed the lowest DTS [Table 4]. The mean DTS for discs containing 0%, 2.5%, 5.0%, and 10.0% CPC was 512.94 ± 86.93, 209.72 ± 42.97, 206.01 ± 29.89, and 181.16 ± 34.69 MPa, respectively [Table 4]. There was a significant difference between the control group and the groups containing 2.5%, 5.0%, and 10.0% CPC. There was no statistical difference among discs containing 2.5%, 5.0%, and 10.0%



**Figure 5:** Close-up view of specimen in place while testing diametral tensile strength

**Table 3: Amount of cetylpyridinium chloride release from the acrylic discs**

	Day 7 (a)	Day 15 (b)	Day 30 (c)	Day 60 (d)	Day 180 (e)	P	Post hoc pairwise comparison
Group I (n=3)							
Mean	0.00	0.00	0.00	0.00	0.00	-	-
SD	0.00	0.00	0.00	0.00	0.00		
Group II (n=3)							
Mean	0.90	0.79	0.81	0.73	0.46	0.017*	a, b, c, d>e
SD	0.02	0.01	0.01	0.02	0.01		
Group III (n=3)							
Mean	1.42	1.12	1.33	1.41	1.06	0.018*	a, c, d>b, e
SD	0.01	0.01	0.02	0.02	0.02		
Group IV (n=3)							
Mean	1.99	1.63	1.73	1.94	1.79	0.19 NS	-
SD	0.01	0.01	0.01	0.01	0.01		

\*P<0.05. SD: Standard deviation; NS: Not significant

**Table 4: Diametral tensile strength (in MPa) of modified acrylic discs before and after water aging**

	DTS before water aging		DTS after water aging		Mean difference	SD	P <sup>b</sup> , significance
	Mean	SD	Mean	SD			
Group I (n=10)	512.94	86.93	474.76	115.62	38.18	116.66	0.241 NS
Group II (n=10)	209.72	42.97	175.47	34.03	34.25	48.53	0.047*
Group III (n=10)	206.01	29.89	156.77	32.36	49.24	37.18	0.009*
Group IV (n=10)	181.16	34.69	106.73	18.09	74.43	33.98	0.005*
P <sup>a</sup> , Significance	<0.001*		<0.001*				
Post hoc pairwise comparison <sup>c</sup>	Gr1>Gr2, Gr3, Gr4		Gr1>Gr2, Gr3, Gr4				
			Gr1>Gr2>Gr4				
			Gr1>Gr3>Gr4				
			Gr4<Gr3, Gr2<Gr1				

DTS: diametral tensile strength; SD: standard deviation; NS: not significant, <sup>b</sup>Wilcoxon signed-rank test <sup>c</sup>Mann Whitney U Test, \*Significant ( $P < 0.05$ )

CPC. This indicates that the addition of CPC lowers the strength of self-cure acrylic.

The effect of CPC incorporation on long-term stability of the DTS was evaluated by soaking the modified and control acrylic discs in distilled water at 37°C for 180 days. The mean DTS for wet-aged discs containing 0%, 2.5%, 5.0%, and 10.0% CPC was 474.76 ± 115.6, 175.47 ± 34.03, 156.77 ± 32.36, and 106.73 ± 18.09 MPa, respectively [Table 4]. Data analysis with Kruskal–Wallis test and Mann–Whitney *U*-test indicated that the four groups were significantly different ( $P < 0.05$ ). These findings showed a significant difference between group I and the other groups. There was no significant difference between groups II and III. Mann–Whitney *U*-test confirmed that there was a significant difference ( $P > 0.05$ ) between the strength of aged and unaged specimens.

## Discussion

There is a considerable increase in the total microbial population and an altered microflora in the presence of orthodontic appliances. To reduce the bacterial load, there should be a controlled and sustained release system. Hence, in this study, the acrylic resin itself was modified by the addition of CPC in concentrations of 2.5%, 5%, and 10% by weight. CPC is an effective antiplaque agent regulated by the Food and Drug Administration (FDA) as an over-the-counter drug and widely used in oral hygiene aids. In a rule effective April 2, 2004, the FDA amended the food additive regulations to permit CPC as an antimicrobial agent. Its safety and efficacy have been evaluated extensively and proven based on cytotoxicity data collected from many animal studies.<sup>[4]</sup>

In this study, an initial burst of CPC release was observed from the modified acrylic discs ranging from 0.9 g/mL for 2.5% CPC group to 1.99 g/mL for 10% CPC group. Then levels in solution showed a general decreasing trend till 180 days. However, despite the reduced level of CPC released in solution, the amount of CPC remaining in the modified discs inhibited growth of *S. mutans* over

a 196-day period as evident by the zone of inhibition around these discs.

The ability of the modified acrylic to release adequate amounts of CPC over a long period is an important characteristic for clinical benefits that was observed in this study. To our knowledge, no other studies in literature have evaluated acrylic modified with CPC, and hence a direct comparison of our results is not possible. But Al-Musallam *et al.*<sup>[9]</sup> evaluated the effect of a modified orthodontic adhesive resin against *S. mutans*. They used CPC in the same concentrations: 2.5%, 5% and 10%. They found that CPC was released from the modified adhesive discs and the amount increased with increase in the concentrations, which agrees with the results of this study.

In this *in vitro* study, the modified acrylic discs were subjected to disc diffusion assay to test their efficacy against *S. mutans*. Except for the control group, all the samples showed inhibition of bacterial growth around them. The amount of inhibition increased with an increase in the concentration of CPC, similar to the study done by Al-Musallam *et al.*<sup>[9]</sup> The samples were water-aged for 196 days and tested for their antimicrobial activity every 14<sup>th</sup> day. All samples containing CPC showed bacterial growth inhibition. The inhibition zone was highest around the discs at day 1, but the efficacy decreased with the increase in time of water aging. The possible reason for this could be the leaching of the CPC with time when the discs were water-aged.

Lobene and Soparker<sup>[10]</sup> studied various agents for antiplaque activity. When used without mechanical plaque control only alexidine, pyrometallic acids and CPC showed antiplaque activity among all. Roberts and Addy<sup>[11]</sup> compared the antimicrobial property of four cationic antiseptics in different *in vivo* and *in vitro* conditions. When tested on BHI broth, chlorhexidinegluconate and alexidine were found active against all test bacteria even at low concentrations, but the same inhibitory property was shown by CPC at a relatively high concentration. In the presence of yeast and food extract, chlorhexidinegluconate, CPC, and alexidine showed the antibacterial property. On

addition of human serum, CPC showed the maximum inhibition of bacterial growth.

In contrast, Herrera *et al.*<sup>[12]</sup> in a 3-month randomized clinical trial to assess clinical and microbiological effects of a CPC dentifrice and mouth rinse in orthodontic patients concluded that the use of CPC-based toothpaste and mouth rinse in orthodontic patients had limited effect in reducing plaque accumulation and gingival inflammation. The effects were little and highly variable. However, the use of the test products was not associated with adverse effects.

The DTS of all the acrylic discs was evaluated before and after aging. For the unaged discs, there was a significant difference between the control and other three groups; the modified acrylic groups containing 2.5%, 5.0%, and 10.0% CPC were weaker than the control acrylic group. Water-aged discs also showed a similar trend. When DTS was compared between aged and unaged discs, all CPC-containing discs showed a significantly lower tensile strength after water aging. These data indicated that although the CPC imparts antimicrobial activity, it lowers the strength of the acrylic. This finding is similar to the study of Al-Musallam *et al.*<sup>[9]</sup> who found that water-aged adhesive discs showed lower DTS when compared with unaged group.

CPC added to the acrylic made the material more brittle, not more deformable. Addition of CPC did not interfere with the curing of the acrylic. This could have been because it has no long aliphatic chain, and hence it cannot act as a plasticizer. Depending on the hydrophilic-lipophilic balance, CPC might remain essentially entangled in the acrylic.<sup>[9]</sup>

Modified CPC-acrylic has potential advantages, such as prolonged antimicrobial activity, over glass-ionomers, or resin-containing glass-ionomers, which offer only short-term fluoride release unless replenished with fluoride-containing mouthwash or toothpaste.<sup>[13]</sup> In a study done by Moran and Addy<sup>[14]</sup> to check the maximum uptake of chemicals such as alexidine, CPC, chlorhexidine gluconate, and chlorhexidine acetate by polymethyl methacrylate and to understand the pattern of molecular attachment to the recipient surface by construction of adsorption isotherms, it was demonstrated that there was a significantly greater uptake of CPC than other antiseptics.

There are some limitations of this *in vitro* study. The maximum safe level for long-term continuous exposure to CPC is not known. Although no potential side effects have been reported, clinical trials are needed to check the safety of a sustained release system. Our findings showed that addition of CPC reduced the tensile strength of the

acrylic. However, the strength of acrylic would vary with its thickness, so the modified acrylic resin used in this study should also be tested to determine its suitability for clinical use.

Further research is needed before self-cure acrylic with antimicrobial additives can be developed into a clinically and commercially useful product.

## Conclusion

- CPC showed a sustained release when added to self-cure acrylic resin over a period of 196 days
- Addition of CPC imparted the antimicrobial property to the acrylic
- Addition of CPC lowered its DTS but did not interfere with the curing of the acrylic.

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## Conflicts of interest

There are no conflicts of interest.

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