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Designing an antibacterial acrylic resin using the cosolvent method —Effect of ethanol on the optical and mechanical properties of a cold-cure acrylic resin

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Antimicrobial cetylpyridinium chloride (CPC) has low miscibility with acrylic resin monomer but can be homogeneously mixed using ethanol as a cosolvent. This study investigated the effects of ethanol addition on the properties of a cold-cure acrylic resin. Ethanol was an excellent cosolvent for CPC and methyl methacrylate monomer (MMA), but the cured resin exhibited a strong change in coloration to yellow (ΔE^*_{ab}>8) and a drastically reduced bending strength (from 97 to 25 MPa) and elastic modulus (from 2.7 to 0.6 GPa) when equal volumes of ethanol and monomer were used together, possibly due to the solvation and deactivation of radicals by ethanol. However, these unfavorable effects diminished when the ethanol/MMA ratio was reduced to 0.25, and became smaller when each specimen was depressurized and excess ethanol was removed. Thus, it may be possible to develop a molecularly uniform antibacterial acrylic resin with acceptable color and strength using this simple technique.

Keywords: Acrylic resin, Antibacterial agents, Uniform mixing, Cosolvent, Ethanol

INTRODUCTION

Acrylic resin is an extremely useful and versatile dental material because of its easy handling, excellent performance, and reasonable cost. Polymethyl methacrylate (PMMA) is the most popular type of resin and is widely used as a base, rebase, or relining material for dentures, as a base for some orthodontic appliances, and as adhesive cement[1]. Recently, various antibacterial dental materials have been developed to reduce the risk of infection from unhygienic dental devices to the elderly or people with suppressed immunity[2]. Therefore, it is important that we develop optimal methods for effectively and safely imparting a sustainable antibacterial function on dental materials according to their properties.

Generally, there are two major methods of functionalizing the surface of dental materials. The first is to alter the surface by coating it with appropriate agents or through chemical modification of the near-surface layer[3]. The advantage of this approach is that the alteration is limited to the thin surface region; therefore, it does not affect any bulk material properties. However, the drawback is that the imparted function on the surface may be easily lost through physical, chemical, or mechanical damage. The second approach aims to functionalize the entire body by uniformly mixing the modifying agents with the basic body material[4-6]. With this approach, one must be careful not to disrupt any original properties of the body material, but it does have the advantage that even if the functionalized surface is damaged, a fresh surface emerges recursively. This self-renewal property may be advantageous for denture base-related materials[5,6], which are sometimes re-adjusted by grinding.

When using the latter approach, it is crucial that homogeneity is maintained in the modified material to retain its mechanical properties. In the case of adding powder-like antimicrobial agents to acrylic resin, the mixing order is important. Cationic surfactants with a quaternary ammonium head group [*e.g.*, cetylpyridinium chloride (CPC) and cetyltrimethylammonium chloride (CTAC)] are well-known antimicrobial agents that are supplied as a fine powder. Therefore, it would be fair to assume that modified resin could be prepared by simply adding the agent/resin powder mixtures to the resin monomer. However, it is not clear whether these different powders are truly mixed uniformly. Furthermore, both CPC and CTAC are insoluble in monomers with a low dielectric constant [ε_r=6.4 for methyl methacrylate monomer (MMA) *vs.* 80.2 for water[7]]; therefore, although the CPC or CTAC particles appear dispersed, the internal structure of the cured body is essentially heterogeneous, with no special interconnection between the additive particle and the resin phases, *i.e.*, the particles are simply there "as is".

This situation can be rectified by using a cosolvent[8,9] that dissolves both of the incompatible components as shown in Fig. 1. It is known that some polar aprotic organic liquids serve as good cosolvent of CPC or CTAC and MMA. Among them, we chose ethanol because of the following reasons. In comparison to acetone, which is also a good cosolvent, ethanol is less harmful to the oral tissues and is actually used as a liquid component of tissue conditioner. While ethanol does not contribute to polymerization, it can be removed from the system as easily as acetone by moderate heating because it is volatile. Hydroxyethyl methacrylate (HEMA) is a hydrophilic comonomer of MMA and is another good cosolvent, but it might cause extra water sorption in the prepared resin. Preliminary experiments have shown that both CPC and CTAC are highly soluble in ethanol

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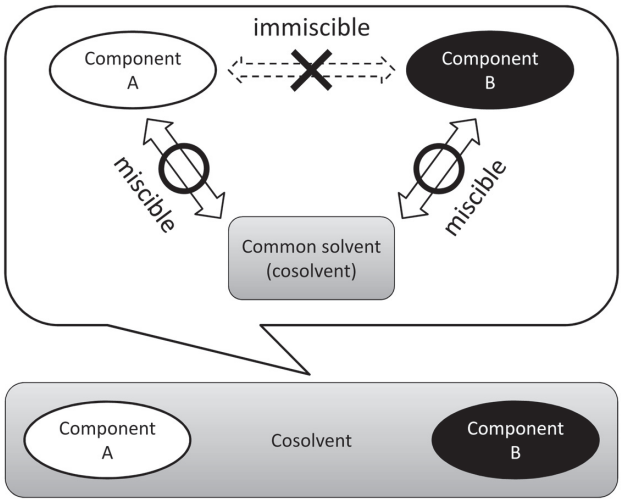

Fig. 1 The mixing of incompatible components using a common solvent.

over 10 wt% and that MMA (ε_r=6.4) is miscible with ethanol (ε_r=25.3[7]) at any ratio. Thus, ethanol may be an extremely promising cosolvent for these components, allowing the preparation of a new hygienic acrylic resin using this simple method. It is also known, however, that alcoholic compounds retard or inhibit radical reactions by acting as a hydrogen donor to the radicals[10].

Therefore, in this study, the basic mechanical (bending strength and elastic modulus), optical (CIE $L^*a^*b^*$ and Raman spectrum), and visual (fractography) properties of an ethanol-containing cold-cure acrylic resin were investigated, and an appropriate content of ethanol in the acrylic resin was assessed. This type of resin can be used as a host material for antimicrobial agents; therefore, these findings will significantly advance the development of a new self-renewal hygienic denture base acrylic resin using the "cosolvent" method.

MATERIALS AND METHODS

Materials

A multi-purpose cold-cure acrylic resin (PROCAST DSP clear shade, GC, Tokyo, Japan) was used, which consisted of a liquid component (MMA with a small amount of accelerator) and a powder component (fine PMMA spheres with a small amount of initiator on them). In the following sections, these liquid and powder components are referred to as MMA and PMMA, respectively. Ethanol (special grade, Wako, Osaka, Japan) was used as an MMA-compatible solvent.

Sample preparation

Following the manufacturer's instructions, a powder-to-liquid (P/L or PMMA/MMA) ratio of 10 g/5 mL was chosen, and this ratio was kept constant. In addition to the standard preparation using MMA alone (0E), ethanol-added MMA was also used as the liquid, in which ethanol was added at either a quarter volume (0.25E) or equivolume (1E) to the fixed amount of MMA. The ethanol-to-MMA ratio will be denoted by E/M hereafter. Large E/M value of 1 intended excessive ethanol content in order to look into any adverse effects. In contrast, the E/M value as low as 0.25 assumed a possible smallest amount in dissolving antimicrobial agents.

PMMA was added to MMA or ethanol-added MMA, and the mixture was vibrated to homogenize it and to remove the trapped air. The slurry was poured into a silicone-rubber open mold and pressed using a pair of glass plates to fabricate the specimen to a dimension of 40×10×2 mm. The setup was then maintained at 45°C (manufacturer's recommendation: 40–50°C) and 0.25 MPa for 30 min to enhance curing. Following preparation, each specimen was kept at room temperature in an atmospheric environment for 12 h and was then depressurized by 98.8 kPa (*i.e.*, to 2.5 kPa) and maintained at that pressure for up to 45 days to eliminate the residual ethanol. To determine the effect of the trace amount of residual ethanol on the properties of the resin, the usual water-immersion step was omitted in this experiment.

Color measurement

The color of the cured specimens was measured based on the CIE $L^*a^*b^*$ method using a spectrophotometer (CM-2002, Konica-Minolta, Tokyo, Japan). Each specimen was placed on a white background plate and observed using a 10° field of view and illuminant D65 with the specular component excluded (SCE) mode. These conditions were selected to mimic the ordinary view of an entire denture base under natural lighting. Each measurement was repeated three times.

Bending test

A three-point bending test was performed on each specimen using a universal mechanical testing machine (AG-IS 20kN, Shimadzu, Kyoto, Japan), with an inter-fulcrum distance of 20.0 mm and a crosshead speed of 1 mm/min. Load-displacement curves were then plotted to measure bending strength, elastic modulus, and toughness.

Scanning electron microscopy (SEM) observation

Following the bending test, the fracture surface of the specimens was observed by SEM (SSX-550, Shimadzu). Each specimen was gold-sputtered at a thickness of 20–50 nm using a quick coater (SC-701AT, Sanyu Electron, Tokyo, Japan), and secondary electron (SE) images were then observed at a low magnification with an acceleration voltage as low as 5 kV to avoid burnout of the resin specimen surface.

Raman spectrum measurement

To identify the local chemical structure of the resin component and to detect the trace amount of ethanol, Raman spectra were obtained in the wavenumber range for the polymerizing group ($H_2C{=}C<$) and alcohol group (C–OH) using a microscopic laser Raman

spectrophotometer (NR-1800, Jasco, Tokyo, Japan) equipped with an Ar^+ laser (excited at 514.5 nm). (Note: For the polymerizing group, a characteristic C=C stretching band at 1,640 cm^{-1} is generally assessed; however, in the present study, another band of symmetric stretching of the CH_2 in the H_2C=C< group at 2,937 cm^{-1} was examined instead because the change was clearer.) The spectral intensity was normalized in reference to the peak that was not influenced by polymerization.

Statistical analyses

The significance of differences in the color measurements and mechanical tests between conditions was examined using two-way ANOVA, with ethanol content and elapsed time included as factors. Tukey's HSD test was then used to determine where any significance lay. A significance level of α=0.01 was used (n=3).

RESULTS

Color of the resin specimens

The MMA and ethanol mixture was a colorless liquid regardless of the ethanol content. However, when PMMA powder was added to the liquid mixture, the color immediately changed, with the degree of change depending on the ethanol ratio. Figure 2 shows a^* and b^* values for the cured resin with different ethanol contents measured after 12 h and 45 days. Resin with a higher E/M ratio exhibited a significant change in color to pale orange, with the increase in b^* (yellow) being much larger than the increase in a^* (red). This color became deeper with increasing time and following polymerization. However, after 45 days of depressurization, the a^* value decreased to almost half that at 12 h, whereas the b^* value decreased by nearly 10%. This change in color could be perceived with the naked eye.

Figure 3 shows a plot of chroma ($C^*=\sqrt{(a^*)^2+(b^*)^2}$) and color difference ($\Delta E^*_{ab}=\sqrt{(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2}$) against E/M value. Both C^* and ΔE^*_{ab} became significantly larger at higher E/M ratios (p<0.01), with a ΔE^*_{ab} value of 8.5 for 1E but only 2.8 for 0.25E, which was not significantly different from 0E. The C^* and ΔE^*_{ab} values decreased to some degree after the 45-day depressurization.

Mechanical properties of the preparations

In the flexure test, both 0E and 0.25E specimens broke apart abruptly, and quite a short plateau was seen in the load-deflection curve for 0.25E specimens (data not shown). By contrast, 1E specimens also broke, but this took the form of a sharp bend without separation. The load-deflection curve for 1E specimens always included a long plateau followed by a distinct decrease.

Figures 4 and 5 show the bending strength and elastic modulus, respectively, of the prepared specimens. In the absence of ethanol (0E), the bending strength was nearly 100 MPa and the elastic modulus was about 2.7 GPa, which are comparable to previously reported values[11,12]. However, both the bending strength and the elastic modulus significantly decreased with an increasing E/M ratio (p<0.05), with the values for both measurements decreasing by less than 10% in 0.25E specimens, compared with up to 80% in 1E specimens. Furthermore, both the bending strength and the elastic modulus were lower at 12 h than at 45 days, with this difference being particularly large for 1E, which exhibited a nearly 80% reduction at 12 h compared with a 50% reduction at 45 days (p<0.01).

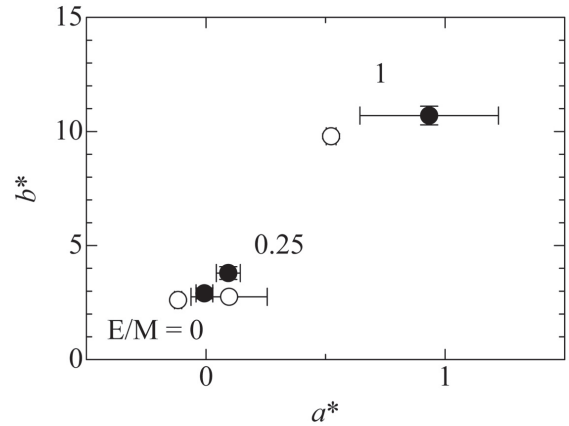

Fig. 2 a^* and b^* values of specimens with different E/M ratios after 12 h (filled shapes) and 45 days (open shapes).

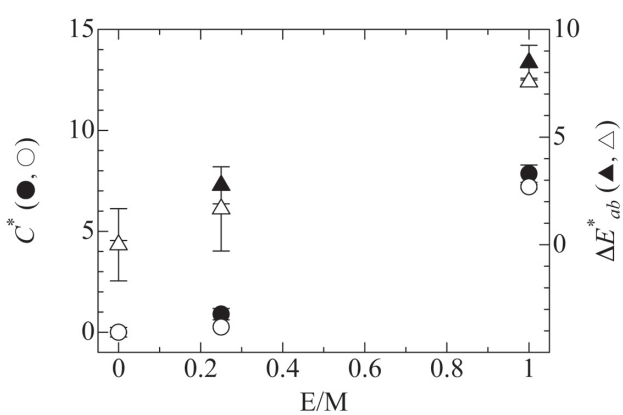

Fig. 3 Chroma (circles) and color difference (triangles) of specimens with different E/M ratios after 12 h (filled shapes) and 45 days (open shapes).

Morphology of the fracture surface

SEM images of the fracture surfaces of 0E and 1E specimens following the bending test 12 h and 45 days after the preparation are shown in Figs. 6 and 7, respectively.

The mode of fracture appeared to differ between the ethanol-free (0E) and ethanol-rich (1E) preparations before the long evacuation (12 h). 0E specimens exhibited

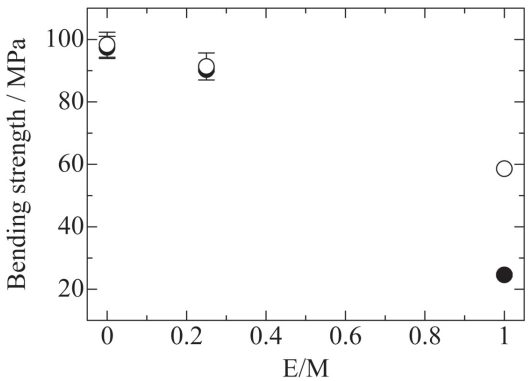

Fig. 4 Bending strength of specimens with different E/M ratios after 12 h (filled circles) and 45 days (open circles).
Standard deviation values are very small for some points [for the 12-h data at E/M=0.25 (sd=0.7) and 1 (0.6), and for the 45-days data at E/M=1 (0.3)].

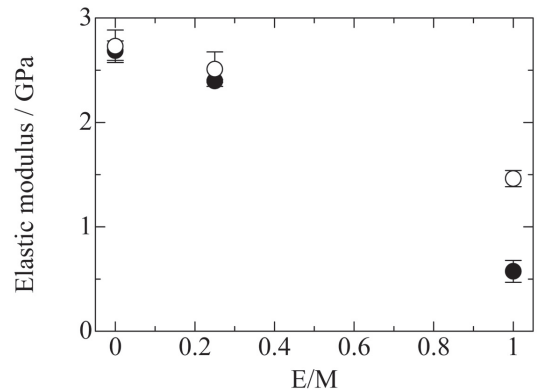

Fig. 5 Elastic modulus of specimens with different E/M ratios after 12 h (filled circles) and 45 days (open circles).

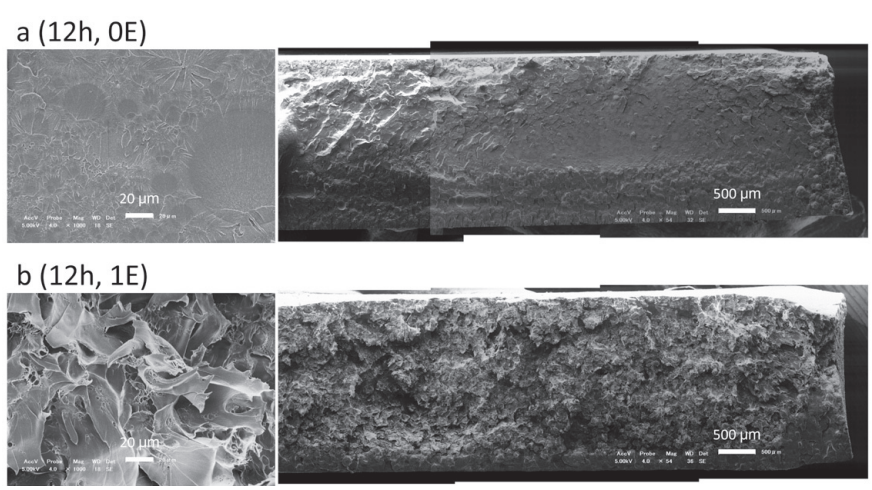

Fig. 6 High (left) and low (right) magnification SEM image of the fracture surface of (a) E/M=0 specimens and (b) E/M=1 specimens after 12 h.

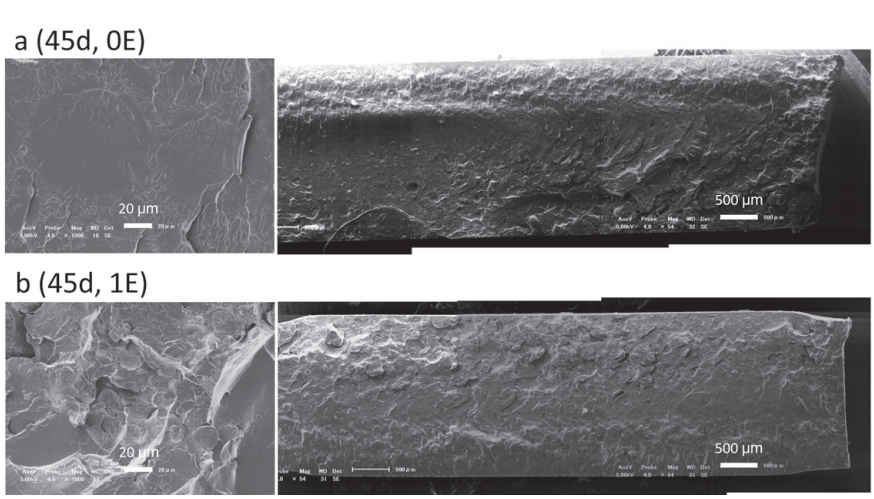

Fig. 7 High (left) and low (right) magnification SEM image of the fracture surface of (a) E/M=0 specimens and (b) E/M=1 specimens after 45 days.

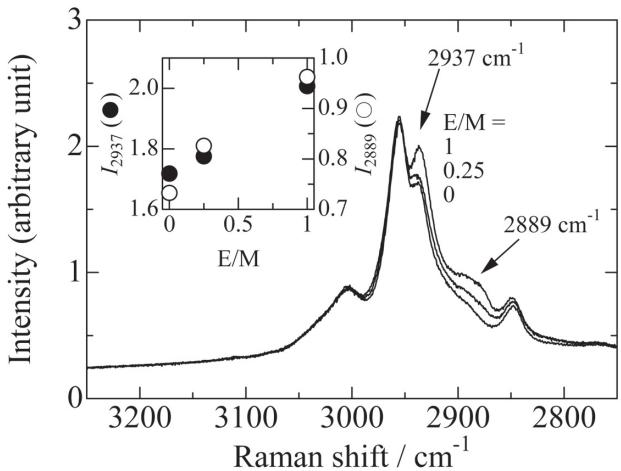

Fig. 8 Raman spectra (CH_2 of the polymerizing group) of specimens with different E/M ratios after 12 h. The E/M dependence of the specific peak intensity is shown in the inset.

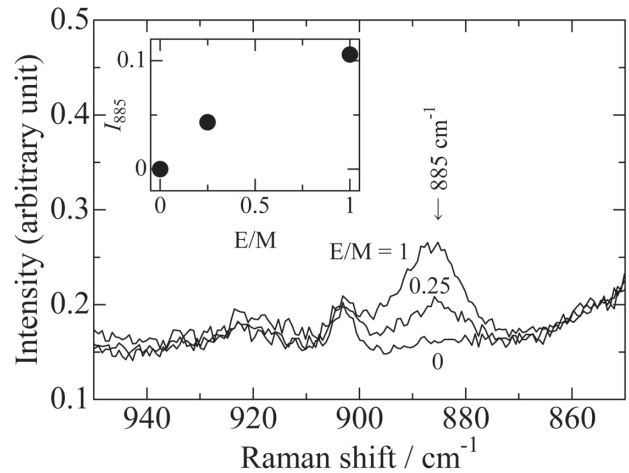

Fig. 9 Raman spectra (C–O of the C–OH in ethanol) of specimens with different E/M ratios after 12 h. The E/M dependence of the specific peak intensity is shown in the inset.

a relatively smooth fracture surface, with a flat, fine, leaf-vein pattern (Fig. 5a), which is typical of the brittle fracture mode. By contrast, 1E specimens exhibited petal-like structures or turned-up thin films with many holes (Fig. 6b), which indicated a ductile fracture mode, *i.e.*, the preparation was more plastic for the ethanol-rich condition.

Following evacuation (45 days), the fracture mode was relatively similar regardless of the ethanol content, with a brittle fracture appearance in all instances. This indicated that the plasticizing effect of ethanol had been diminished following the 45-day depressurization.

Raman spectra for specimens cured with and without ethanol

Figures 8 and 9 show the Raman spectra of the specimens in the wavenumber range for the polymerizing group ($H_2C{=}C<$) and alcohol group (C–OH), respectively. The intensity of the "monomer" band at 2,937 cm^{-1} was stronger in specimens with a higher E/M ratio at 12 h (Fig. 8). However, at 45 days, the spectra were all the same as 0E specimens at 12 h, regardless of their E/M ratios, *i.e.*, the monomer band was absent. This indicated that the polymerization reaction had been completed in 45 days. The intensity of a band relating to the C–O stretching in ethanol also varied depending on the E/M ratio after 12 h (Fig. 9), indicating that the added ethanol remained in the cured specimens. However, no band was seen in the same region after 45 days, showing that the residual ethanol had been lost through the long period of depressurization. This is consistent with our expectation that volatile ethanol can be removed, as described in the introduction.

DISCUSSION

Discoloration of acrylic resin cured in the presence of ethanol

Specimens containing a high ethanol content exhibited a marked color change to yellow compared with the standard preparation without ethanol. However, this change was almost undetectable at an E/M ratio of 0.25 (20v% in the liquid component). It is notable that this color change occurred just after the ethanol-MMA solution was mixed with PMMA, both of which are colorless; was not seen when an accelerator-free MMA of reagent grade was used and heat-cured or when the ethanol content was sufficiently low; and slightly decreased after the residual ethanol had been removed by a 45-day evacuation. These observations suggest that this color change is related to solvatochromism[13] because of some intermediate radical species of an amine accelerator possibly being solvated by ethanol.

Because it is almost impossible to perceive the difference in two colors with a ΔE^*_{ab} value <3[14], the color change in the acrylic resin that contained only a small amount of ethanol (E/M=0.25) may be negligible in comparison to the standard (ethanol-free) preparation. Therefore, the addition of only a small amount of ethanol to dissolve the antimicrobial agent is fairly acceptable in terms of the discoloration.

Effects of ethanol on the mechanical properties of the cured resin

Both the bending strength and elastic modulus of the preparation were smaller at higher E/M ratio. However, these reductions were partially recovered following a long period of depressurization, and the Raman spectral observation showed that the residual ethanol had disappeared after the evacuation. This suggests that the

added ethanol had a plasticizing effect. Similarly, the residual monomer, as detected by the Raman spectra, may also have acted as a plasticizer at an early stage.

The E/M-dependent degradation of the mechanical properties before the evacuation indicated that the added ethanol caused insufficient polymerization. This seems to be supported by the appearance of ductile fracturing at the high E/M ratio of 1. There are two possible explanations for this. First, it is possible that the radical was deactivated by ethanol, suppressing the monomer-to-polymer conversion. This was partially supported by the Raman spectra, in which higher levels of residual monomer were detected in the higher E/M preparations for the 12-h group. However, it is also possible that the radical reaction was terminated before sufficient propagation reaction had occurred because of the resin (mixture of monomer liquid and polymer powder) having a lower viscosity due to the ethanol acting as a diluent, *i.e.*, the degree of polymerization remained low, causing the cohesion energy between the formed PMMA molecules to become lower than that between the usually formed high polymers, which in turn led to lower mechanical properties. The appearance of the fracture surface is consistent with this hypothesis, with the appearance of ductile fracturing in the preparation with ethanol (E/M=1) indicating insufficient polymerization compared with the ethanol-free preparation, which exhibited brittle fracturing.

It should also be noted that the inferior polymerization may have resulted from the addition of ethanol having a "dilution effect" on the composition of the polymerization initiators. However, this may not be an issue because the high ethanol content used in this study was purposefully in excess to determine the optimum amount required to fully mix the microbial agent with MMA. Furthermore, to avoid the unfavorable effects of ethanol, it may be advisable to remove it from the homogeneous mixture of CPC and resin monomer before the polymerization process in some way (Dr. SUGIURA, private communication). Although this approach will have some difficulties (*e.g.*, the re-precipitation of CPC from the MMA phase and the selective removal of ethanol), it is worth attempting in the future.

Although the mechanical strengths became rather low at an E/M value as high as 1, they were fairly acceptable at a lower E/M value of 0.25. Furthermore, once the residual ethanol had been thoroughly removed, the decrease was as small as 10%, and this should become even smaller if the ethanol content can be reduced further. Since the solubility of some antimicrobial agents such as CPC and CTAC is very high in ethanol, as described in the introduction, further reduction in E/M is highly possible. Thus, in terms of the mechanical properties of the resin, the addition of a small amount of ethanol may be suitable as a cosolvent for dissolving antimicrobial compounds in acrylic resin uniformly.

This simple method would be practical in clinic because antibacterial function could be imparted to a variety of denture-related resin easily without seriously affecting the color and the mechanical properties required to the prostheses. To confirm its utility, however, more work should be done further regarding, for example, (1) the actual preparation of resin containing antimicrobial agents with this method, and (2) the confirmation of its antibacterial action against some bacteria as *Candida albicans*, which is a typical indigenous one, yet harmful to people with poor immunity.

CONCLUSIONS

This study investigated the effect of ethanol addition on the properties of a cold-cure acrylic resin. It was found that although ethanol is an excellent cosolvent for uniformly mixing the resin monomer and antimicrobial agent for the preparation of a self-renewal hygienic resin, the addition of ethanol resulted in the following effects:

1) Caused a significant color change to yellow, possibly due to some intermediate radical species of an amine accelerator being solvated by ethanol.
2) Markedly lowered both the bending strength and elastic modulus, possibly as a result of the ethanol (and residual monomer) either acting as a plasticizer in the cured material or causing insufficient polymerization by retarding the monomer-to-polymer conversion or by lowering the degree of polymerization.

However, by limiting the volume fraction of ethanol to the resin monomer to 0.25, these unfavorable effects were almost suppressed. Therefore, it is highly probable that a truly homogeneous antibacterial acrylic resin could be successfully prepared using only a small amount of ethanol as a cosolvent for the antimicrobial (*e.g.*, CPC or CTAC) and the monomer (MMA).

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