To Compare Influence of Commonly Used Food Colourants on Colour Stability of Different Heat Cure Denture Base Resins: An In Vitro Study

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Abstract
With increase in demand of aesthetically pleasing denture prosthesis colour stability is one of most important character of denture. Patients are now more demanding and need of colour stable denture base is need of time. The food and beverages which are consumed by us cause colour change of denture base. In this study two PMMA denture base material (DPI and Trevalon HI) and one polyamide denture base material (M Flex) were used to analyse effect of tea, coffee and turmeric on them at ten, twenty and thirty days.

Forty samples of each denture base materials were made of diameter twenty-five millimetre and width of three millimetre. Samples were divided into sub groups of ten samples each and immersed in solutions of tea, coffee and turmeric. Distilled water was taken as control.

Spectrophotometric analysis of samples was done before immersion in solutions(T₀) and after immersion in solutions at time interval of ten days(T₁₀), twenty days(T₂₀) and 30days(T₃₀).

Results showed there was statistically significant difference between all three denture bases at all time intervals with Mflex showing maximum colour change and Trevalon HI showing minimum colour change. Of all the staining solutions used turmeric caused maximum colour change in all the materials followed by coffee, tea and distilled water respectively.

Keywords: DPI, Trevalon HI, Mflex, spectrophotometer.
Introduction

Colour stability is one of the essential physical property of the denture base resins that help them to retain the originality of different denture base materials. Discolouration of the denture bases can be due to intrinsic and extrinsic factors. Discolouration can cause serious aesthetic problems.

The colour stability of all denture base resins is affected by food and beverages consumed by an individual on daily bases. Tea, coffee and turmeric are most common discolouring agents found in Indian food. The purpose of this study was to evaluate colour stability of three different denture base resins by subjecting them to solutions of tea, coffee and turmeric at different time intervals.

Aims and Objectives

The aim of study was to evaluate the colour change of different types of denture base resins when they come in contact with tea, coffee and turmeric solutions at various time intervals. The objectives of this study were to evaluate colour stability of two types of PMMA and one polyamide heat cure denture base materials when they come in contact with tea, coffee and turmeric solutions at various time intervals and to compare the colour stability among these three denture base resins subjected to common food colourants at different time intervals.

Material and methods

In this study two types of heat cure polymethyl meth acrylate (PMMA) and one brand of polyamide was used. Of the two PMMA materials one was conventional PMMA with brand name DPI (Dental Products of India Burmah Trading Co Ltd) and other was high strength PMMA with brand name Trevalon HI (Dentsply India Pvt. Ltd) was used for making samples. For polyamide Mflex (Macro Dental World Pvt Ltd Ludhiana India) was used. For making samples first wax pattern were made in metal die (Figure 1) with a diameter of twenty-five millimeter and thickness of three millimeter.

Thus, the samples obtained had dimensions of wax pattern (Figure 2a,2b). Samples from PMMA materials were made by compression moulding technique using conventional flasks (Figure 3) and long curing cycle was used. For polyamide material injection moulding technique was used in flasks (Figure 3) specially designed for injection moulding technique. Forty samples of each brands were finished with denture trimming burs and polishing was done by sandpapering, wet buffing and in the end by dry buffing.

For making solutions of colouring agents commonly used brands of turmeric (Catch Shakti Mulla Patti India), coffee (Nescafe Hindustan Unilever Ltd Mysore India) and tea (Brooke Bond Red Label Hindustan Unilever Ltd Mumbai India) were used. Solutions of turmeric, coffee and tea were prepared by adding six grams of colourant to six hundred milliliters of distilled water (Zydus Cadila pharma Pvt Ltd India).

Solutions were stirred and allowed to cool for ten minutes and were filtered through filter paper. Each solution was divided into three parts of two hundred millilitres each so that ten specimens of each brand of denture base resins were immersed into specific solutions.

Figure 1: Metal Die
All three denture base brands were divided into Group A, Group B and Group C consisting of forty samples each made from DPI heat cure denture base, Trevalon HI heat cure denture base and Mflex polyamide denture base resin. They were further divided into four sub-groups of ten samples each to evaluate colour stability. The Groups and Subgroups are shown in (Table 1).

All the samples from each denture base resins were immersed in distilled water for twenty-four hours. Thereafter all samples were dried. On the side of samples away from spectrophotometer, marking was made according to sub group in which the samples were segregated. This marked segregation helped us to record the reading of samples without mixing the samples in each subgroup. Then each sample of resin was subjected to colour measurement using spectrophotometer (Figure 4) before immersion($T_0$) into beverages and food colourant.
After initial measurement samples were immersed into specific solutions and containers of solutions were placed in the incubator at thirty-seven degree Celsius to simulate the conditions in oral cavity and the solution was changed after ten and twenty days. The solutions were stirred every day. Colour measurements were made on tenth (T10), twentieth (T20) and thirtieth (T30) day. The specimens were rinsed with distilled water for five minutes and blotted dry with tissue paper before colour measurement. Colour of each sample was measured using spectrophotometer (RF-5301 pc SHIMADZU, Japan) at each specified time interval (T0, T10, T20 & T30). The mean values of ΔL, Δa, Δb after three measurements were automatically calculated by the spectrophotometer and recorded. Colour difference was calculated from the mean ΔL, Δa, Δb values for each sample with formula: ΔE= (ΔL²+Δa²+Δb²)¹/² where ΔL, Δa, Δb are the differences in L, a, b values before(T0) and after immersion at each time interval (T10, T20 & T30). The repeatability of measurements was evaluated by computing the mean and standard deviation of 10 repeated measurements of selected samples. The mean and standard deviation estimated from the samples for each subgroup was statically analysed. Figure 6 shows colour change of samples after thirty days of immersion in different solutions.

Table 1: Grouping and Subgrouping of samples in different solutions.

<table>
<thead>
<tr>
<th>Group</th>
<th>Material</th>
<th>Sub Group</th>
<th>Solution</th>
<th>No of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Conventional PMMA denture base resin (DPI)</td>
<td>A1</td>
<td>DW (c)</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A2</td>
<td>DW+ Turmeric</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A3</td>
<td>DW+ Coffee</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A4</td>
<td>DW+ Tea</td>
<td>10</td>
</tr>
<tr>
<td>B</td>
<td>High Strength PMMA denture base resin (Trevalon HI)</td>
<td>B1</td>
<td>DW (c)</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B2</td>
<td>DW+ Turmeric</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B3</td>
<td>DW+ Coffee</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B4</td>
<td>DW+ Tea</td>
<td>10</td>
</tr>
<tr>
<td>C</td>
<td>Polyamide denture base resin (Mflex)</td>
<td>C1</td>
<td>DW (c)</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C2</td>
<td>DW+ Turmeric</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C3</td>
<td>DW+ Coffee</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C4</td>
<td>DW+ Tea</td>
<td>10</td>
</tr>
</tbody>
</table>

n = Number of samples; DW= Distilled Water; c= Control Group

The data collected for colour change were evaluated and compared for different groups using Mean values, One-way ANOVA and Multiple post hoc Tucky test.

**Results**

The mean and standard deviation values for all materials after storage in the staining solutions is shown in table -2. A one-way ANOVA (Table-3) showed significant difference in colour change between different denture resin bases at all time intervals (p<0.05). Figure-6 shows maximum colour change in Group C (Mflex), Group A
(DPI) showed intermittent values and Group B (Trevalon HI) showed least colour change. For all dental resins the colour change showed increasing trend from tenth day to thirtieth day. A significant colour change was seen between different staining solutions in each group of denture base materials at all time intervals (Table-4).

Tuckey Post Hoc multiple comparison test showed that turmeric solution caused the maximum colour change in all denture base materials which was significantly higher than colour change caused by distilled water, tea and coffee taken together (p<0.05). Figure 7 shows maximum colour change was caused by turmeric solution followed by coffee, followed by tea solution and in the end distilled water.

Table 2: Mean and Standard Deviation values of all materials.

<table>
<thead>
<tr>
<th></th>
<th>DPI</th>
<th>Travelon HI</th>
<th>Mflex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled Water</td>
<td>10 Days</td>
<td>2.1±1.12</td>
<td>1.8±1.18</td>
</tr>
<tr>
<td></td>
<td>20 Days</td>
<td>2.6±1.40</td>
<td>2.50±1.36</td>
</tr>
<tr>
<td></td>
<td>30 Days</td>
<td>14.7±1.48</td>
<td>9.95±2.83</td>
</tr>
<tr>
<td>Turmeric</td>
<td>10 Days</td>
<td>17.4±3.68</td>
<td>14.83±3.67</td>
</tr>
<tr>
<td></td>
<td>20 Days</td>
<td>20.9±4.48</td>
<td>19.28±4.07</td>
</tr>
<tr>
<td></td>
<td>30 Days</td>
<td>2.3±1.04</td>
<td>2.85±2.39</td>
</tr>
<tr>
<td>Coffee</td>
<td>10 Days</td>
<td>3.3±1.27</td>
<td>4.24±2.73</td>
</tr>
<tr>
<td></td>
<td>20 Days</td>
<td>4.8±1.80</td>
<td>4.99±2.46</td>
</tr>
<tr>
<td></td>
<td>30 Days</td>
<td>1.6±0.72</td>
<td>2.32±1.15</td>
</tr>
</tbody>
</table>

Table 3: ANOVA table for comparison of different solutions on denture base resins at various time intervals.

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>Df</th>
<th>Mean Square</th>
<th>F value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 Days Between Groups</td>
<td>1174.96</td>
<td>3.00</td>
<td>391.65</td>
<td>12.18</td>
<td>0.00</td>
</tr>
<tr>
<td>20 Days Between Groups</td>
<td>1705.09</td>
<td>3.00</td>
<td>568.36</td>
<td>17.55</td>
<td>0.00</td>
</tr>
<tr>
<td>30 Days Between Groups</td>
<td>2644.70</td>
<td>3.00</td>
<td>881.57</td>
<td>26.33</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table 4: ANOVA table for comparison of different solutions on denture base resins at different time intervals.

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>Df</th>
<th>Mean Square</th>
<th>F value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPI 10 Days Between Groups</td>
<td>1281.961</td>
<td>3</td>
<td>427.3</td>
<td>432.5</td>
<td>0.00</td>
</tr>
<tr>
<td>DPI Within</td>
<td>35.566</td>
<td>36</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Groups | 20 Days | Between Groups | 1626.9 | 42 | 542.9 | 115.8 | 0.00 |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Within Groups</td>
<td>168.60</td>
<td>1</td>
<td>4.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30 Days</td>
<td>Between Groups</td>
<td>2270.2</td>
<td>83</td>
<td>756.8</td>
<td>99.7</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Within Groups</td>
<td>273.29</td>
<td>3</td>
<td>6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion

One of the most challenging factors in maintaining aesthetics is the colour stability of the denture base material. The denture base materials come in contact with different beverages and food which have colour changing effect on denture base material. Minimizing colour change is a factor that should be used in the selection of materials and techniques for the best esthetic effect; the material should be translucent. Colour and translucency should be maintained during processing and these resins should not get stained or changed in clinical use. The colour stability criteria may provide important information on the serviceability of materials2.

Figure 6: Representation of color change of different denture base resins at various time intervals
Figure 7: Graph showing staining potential of different staining agents.

In our country people have a habit of drinking a lot of tea and coffee. Turmeric is an important constituent of our food which is found almost in all the Indian dishes. That’s why these three colourants were used in this study. DPI heat cure (Group A), Trevalon -High strength heat cure (Group B) and Mflex polyamide denture base (Group C) materials were taken as colour stability was to be analyzed between conventional PMMA denture base material, high strength PMMA material and polyamide denture base material.

Discolouration of acrylic resins may be caused by several factors. Intrinsic factors such as degree of conversion and residual monomer can influence colour stability. According to Shagsoz NP et al intrinsic discolouration can be due to alteration of the resin matrix and the interface of matrix and fillers. Inhibitors and and quantity of amine involved in polymerization also plays lone in internal discolouration. According to Asmussen E monomer content and peroxide may also play a role in intrinsic discolouration. Extrinsic factors for discolouration include staining by adsorption or absorption of colourants as a result of contamination from exogenous sources. The staining of polymeric materials by coloured solutions, coffee and tea, nicotine, and beverages has been reported in studies carried out by Satau N et al.

Another possible source of colour change is the porosity. Beech DR indicated that residual monomer causes three principle effects: porosity, dimensional change through stress relief, and decreased physical properties. Firtell DN showed in his study that porosity leads to slow influx of oral fluids through the acrylic resin which may cause change in colour due to beverages and different food we eat. Thus, the denture must be nonporous in order to resist staining. Rough surface of denture base may be an important factor in colour change as it directly and indirectly affects staining. Jain V et al stated that restoration with high degree of roughness lead to discolouration of same. Colour change in nylon denture base is more as show in Figure 6. The larger colour change in nylon denture base in comparison to two other acrylic heat cure denture bases is due to higher hygroscopic and water sorption properties. Lai YL et al found that the frequency of amide groups along the chain had affected the water sorption and the chemical properties of each type of nylon. For making samples roughness free laboratory procedure of finishing and polishing was done because according to Chatzivasileiou et al laboratory polishing produced smoothest surface than chair side polishing procedure. Patel SB et al in their study stated that differences in finishing and polishing of nylon materials compared to PMM cause rough surface which are more susceptible to staining. Abuzar et al evaluated the surface roughness of a polyamide denture base material in comparison with PMMA, and found that polyamide specimens produced a rougher surface than PMMA, both before and after the polishing process. The unpolished polyamide surface might have been affected by some degrees of disintegration of the mold surface which was heated to a higher temperature compared to PMMA, and also the pressure during injection molding.
Time interval for measuring colour change was taken at 10th, 20th and 30th day. This time interval was taken as per studies done by Scotti R et al\textsuperscript{14}, according to which as immersion of denture resin in staining solutions approached 30th day, they showed maximum colour change. As immersion time was increased from 30\textsuperscript{th} day denture base resins showed low levels of colour changes, which were not significantly different from colour changes in controls. After 30\textsuperscript{th} day of immersion of samples in solutions a balance in water sorption values was seen in a study conducted by Al Mulla et al\textsuperscript{15}.

Discolouration can be evaluated visually and by using different instruments (spectrophotometer and colourimeter). Colour perception by visual assessment of objects is a subjective, physiologic, and psychologic process that varies between and within persons. Since instrumental use eliminates subjective interpretation of visual colour comparison spectrophotometers have been commonly used to measure colour change in dental materials\textsuperscript{16}.

The commonly used colour system is the CIE-LAB system. In this system the colour of the samples is expressed as three variables, namely L*, a* and b* from which ∆E is calculated. The value of ∆E* represents relative colour changes that an observer might report for the materials after treatment or between time periods. Thus, ∆E* is more meaningful than the individual L*, a*, and b* values as reported by Kuehni et al\textsuperscript{17} and Seghi et al\textsuperscript{18}.

Of all the staining solutions used turmeric showed maximum colour change in all three denture base materials at all the time intervals followed by coffee, tea and distilled water in the end. It has been proposed by Gupta et al\textsuperscript{19} that discolouration from turmeric was due to presence of conjugated diarylheptanoids like curcumin, which is responsible for orange colour and highest staining by turmeric solution.

Staining by coffee and tea solutions is due to presence of tannic acid in them. According to Moon C and Ruyter E\textsuperscript{20} discolouration by tea was due to adsorption of polar colourants from tea whereas discolouration from coffee was due to both the surface adsorption and absorption of colourants. This might be the reason for the samples to be less colour stable in coffee than in tea. Studies conducted by S.V Singh et al\textsuperscript{21} and Faiza Amine et al\textsuperscript{22} also showed maximum colour change in turmeric solution followed by coffee, tea and distilled water.

However, in this vitro study all factors that contribute in the discolouration of acrylic resins like residual unpolymerized monomers resulting from incomplete polymerization, filler and monomer composition, material wear, poor oral hygiene, nutrition and surface roughness could not be assessed. Prosthetic restoration undergoes various harsh influences in the oral environment, various proteins and enzymes are present in saliva, food products and beverages present in the oral environment, variety of food and drink having extremes of temperature, functional and parafunctional loading, smoking and bad oral hygiene. Colour changes were attributed by all these factors. Therefore, to test the oral environment influences on the colour stability of denture base materials, a more comprehensive in vivo study can be explored in future.

**Conclusion**

Within limitation of this invitro study, following conclusions were drawn:

- All the three denture base resins included in the study showed significant colour change in all the three solutions used in the study at various time intervals.
- Poly amide denture base resin exhibited highly significant colour change regardless of any staining solution used at different intervals of time.
High strength denture base showed least colour change and Conventional denture base resin showed intermediate colour change.

Turmeric solution exhibited to cause maximum colour changes followed by coffee and tea in all the denture base resins.

With increase in time there was increase in colour change of all three-denture resin in all the staining solutions.

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