

# The evaluation of the amount and sustain release of antifungal agent clotrimazole incorporated in different polymer matrix in artificial saliva at a pH of 6.2.

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## ABSTRACT

**Background:** Candidiasis in the oral cavity is an opportunistic infections, the most common species involved is *Candida albicans*. Clotrimazole (CTZ) is the first line broad-spectrum antifungal drug that has been widely used for the prophylaxis and treatment of oral and vaginal candidiasis. It is seen that the effect of drug is better locally as compared to oral intake. The aim of the present study is to find out the quantum release of clotrimazole from the incorporated materials in the artificial salivary medium at the pH of 6.2 and to access the physical properties of the clotrimazole incorporated reliner and tissue conditioner. **Methods:** The antifungal clotrimazole is incorporated in two types of denture base material used for this study at 2%, 5% and 10% concentration by weight. The release of clotrimazole out of polymer matrix was studied at various pH. The main property of tensile strength of the specimen before and after the chemical release of the clotrimazole was tested. **Result:** Concentration was maximum on 8th day and uniformly decreased thereafter. The maximum amount of release upto 8th day was 2.52, 4.23, 9.84 mg for 2%, 5% and 10% respectively. **Conclusion:** Topical release of antifungal agents is more effective than systemic intake, which has adverse effect like renal toxicity and CNS toxicity.

**Keywords:** Clotrimazole, *Candida albicans*, kinetics, soft liners, tissue conditioner

## INTRODUCTION

Denture related stomatitis is an inflammatory reaction of the oral tissue that is in contact with the denture.<sup>[1]</sup> The tissue surface of the denture serves as a reservoir for bacteria, fungi and other micro-organism. Candidiasis in the oral cavity is an opportunistic infections, the most common species involved is *Candida albicans*. It becomes infectious when predisposing factors such as antibiotic therapy, diabetes mellitus, corticosteroid therapy, xerostomia (dry mouth), chemo/radiation therapy, and immunosuppression are present.<sup>[2]</sup> Recently the arrival of the HIV (human immunodeficiency virus) infection has stemmed in a reappearance of oral *Candida* infections. The other elements responsible for blossoming of oral candidiasis are general debilitation, poor oral hygiene, and ill-fitted dentures.<sup>[3]</sup> Fungal opportunistic infections mainly oral candidiasis in immunosuppressive patients, are a major cause of morbidity and mortality in cancer patients.

Chronic antimycotic therapy in high doses is disagreeable for treatment of oral infections due to impending side effects. Topical release of antifungal agents is more effective than systemic intake, which bypasses the adverse effect like renal and CNS toxicity.<sup>[4]</sup>

Clotrimazole (CTZ) is the first line broad-spectrum antifungal drug that has been widely used for the prophylaxis and treatment of oral and vaginal candidiasis.<sup>[5]</sup> It is seen that the effect of drug is better locally as compared to oral intake. The only option available for local clotrimazole is in the form of troche (a common brand is Mycelex® troche, Miles Pharma, USA). Therefore, there is a necessity for the improvement of CTZ buccal bioadhesive controlled release formulation.<sup>[6]</sup>

The aim of the present study is to find out the quantum release of clotrimazole from the incorporated materials in the artificial salivary medium at the pH of 6.2 and to access the physical properties of the clotrimazole incorporated reliner and tissue conditioner.

## MATERIALS AND METHODS

### Instruments used in the study were:

A) Instruments used for the standard specimen for auto polymerizing reliner and tissue conditioner with the incorporated clotrimazole of 15 mm length and

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breadth and 2mm of thickness and also the same size of standard plates without antifungal incorporation for the control study.

Porcelain mixing jar, 1 millimeter pipette, metal dental flask with clamps, single pan electronic weighing machine.

B) Steel die with measurement of 15\*15\*2 mm

C) Die stone

D) any other instrument used for routine dewaxing and packing procedures, 100 ml beakers, standard flask, separating funnel and distilled water.

**Fabrication of clotrimazole incorporated specimens**

Impression of the steel dies was taken with die stone and after the die stone has set, it was separated. The die stone mold thus formed was filled with pink modeling wax and then it was invested in brass flask using plaster and stone I 1:1 ratio. After it has set, dewaxing was done to eliminate wax. Then tinfoil substitute was coated over the approximating surface and allowed to dry.

**Incorporation of clotrimazole in acrylic reliner and acrylic tissue conditioner**

The antifungal clotrimazole is incorporated in two types of denture base material used for this study at 2%, 5% and 10% concentration by weight. The accurately weighted powder of 400 gms acrylic polymer of reliner and tissue conditioner are taken separately in a mortar with this accurately weighted raw material of clotrimazole of various concentration of 2%, 5%, 10% weighed and also 1% of sodium lauryl sulphate is also weighted separately using a single pan electronic weighting machine and is mixed thoroughly using the pestle.

With this corresponding liquid like Di-N-butyl phthalate is mixed, liquid volume of in 0.2ml taken by using 1ml pipette. Once dough stage is attained, it should be packed in the space and compressed, interposing wet polyethylene sheet and the excess

flash was removed and the flask was compressed again.

Using the above mentioned procedure 50 specimens were made in each group of the two materials at various concentrations. Then these samples were studied for the quantum of the release of the clotrimazole.

**Chemical release of the clotrimazole from the study specimens by UV spectrophotometer**

The release of clotrimazole out of polymer matrix was studied at various pH, and was maximum at pH 2.5. since the pH of the saliva is near neutral and also due to the fact that candida grows in acidic pH of ranges 5.8-6.2 for diabetic and immunocompromised patients. The release was studied at pH of 6.2, but there was no release of clotrimazole that necessitate the use of enhancer that is sodium lauryl sulphate.

Clotrimazole was mixed with resin and tissue conditioner in the concentration of 2%, 5% and 10% by weight along with 1% sodium lauryl sulphate the enhancer and made into square specimen of 15\*15\*2 mm.

Specimen were kept in separate beaker immersed in 10 ml of artificial saliva at pH 6.2 with 10ml of artificial saliva. 1ml of solution was removed with the pipette at interval of 8 days upto 72 days and tested for clotrimazole concentration by extraction with chloroform and absorption at 268 nm as the hydrochloride. The main property of tensile strength of the specimen before and after the chemical release of the clotrimazole was tested.

**Statistical analysis**

The two groups were analyzed by using students 't' test and p value < 0.001 was considered significant.

**RESULTS**

**Table 1:** Mean amount of clotrimazole release in mg from R, T, in group II, III, IV.

Specimen	8 hrs	16 hrs	24 hrs	32 hrs	48 hrs	56 hrs	64 hrs	72 hrs	Total
<b>Group II</b>									
R	2.52	1.80	1.47	1.10					7.50
T	2.83	1.38	1.62	1.28					7.73
<b>Group III</b>									
R	4.23	3.33	2.71	2.30	1.68	1.55	1.19	0.97	19.99
T	4.31	3.56	3.18	2.71	1.91	1.58	1.17	0.67	20.12
<b>Group IV</b>									
R	10.53								39.63
T	10.36	8.27	6.57	5.28	3.15	2.27	1.76	1.39	43.43

Where

R- auto-polymerizing acrylic reliner

T -autopolymerizing acrylic tissue conditioner

Group I-control

GroupII- 2% incorporation of clotrimazole GroupIII-5% incorporation of clotrimazoleGroupIV-10% incorporation of clotrimazole

**Table 2:** Comparison of mean Cumulative amount of release of clotrimazole in days between R, T, with all concentration with F value and P value using student t test

Specimen	F	P	18 hrs	16 hrs	24 hrs	32 hrs	40 hrs	48 hrs	56 hrs	64 hrs	72 hrs
R	31.162	000	5.72	10.02	15.43	16.48	23.41	26.62	27.96	29.22	29.81
T	37.63	000	5.83	10.35	14.10	17.10	23.79	27.33	29.18	30.01	31.78

**Table 3:** Calculation of p value and f value in association with the mean concentration of various materials

Specimen	F value	P value	2%	5%	10%
R	229.788	0.000	4.92	14.21	30.03
T	273.003	0.000	5.51	13.98	31.34

**Table 4:** Analysis of tensile strength

Specimen	F value	P value	GrI PSI	Gr II PSI	Gr III PSI	Gr IV PSI
R	48.26	0.000	812.34	782	755.72	694
T	28.35	0.000	556.59	537	503.46	450



Figure 1: Spectrophotometer

## DISCUSSION

The frequency and the etiology of denture-induced candidiasis were debated widely in the literature. Denture-induced candidiasis is a multifactorial disease and is ordinarily seen in elderly people. *C. albicans* is the chief causative agent. Other *Candida* species such as *C. glabrata* are commonly identified particularly in immunologically compromised patients.<sup>[7,8]</sup>

The treatment of this ailment is difficult due to its multifactorial etiology. Predisposing factors should be controlled to avert the relapse of fungal infections. Additionally, denture-induced candidiasis is a mixed multi-species biofilm infection which provides further challenges for its management and effective treatment requires disruption of this biofilm.<sup>[9]</sup> Several etiquettes have been recommended for the treatment of patients suffering from oral candidiasis.<sup>[10]</sup> Numerous therapeutic modalities are existing ranging from denture disinfection to

systemic antifungal therapy.<sup>[11]</sup> Despite this the recurrence rate of denture-induced candidiasis is high. It has been proposed that this is due to poor access of the antifungals onto the fitting surface, their poor penetration into the microbial biofilm on the porous denture material, as well as their rapid clearance by saliva and tongue movements.<sup>[12]</sup> Local drug carriers have been suggested to prolong the efficiency of oral treatment in order to maintain ideal therapeutic drug levels at the site of infection over the required period by release of the drug at a predetermined controlled rate.<sup>[13]</sup>



Figure 2: Artificial saliva & Clotrimazole

Direct conveyance of the drug to the site of infection diminishes the risk of systemic side effects or drug-drug interactions. Satisfactory results for incorporation of antifungal agents in different polymeric systems have been stated.<sup>[14]</sup> Clotrimazole is a commonly used disinfectant with a remarkable antifungal, antibacterial and anti-biofilm potency. Suggestively, advent of resistance has not been detected with clotrimazole, but despite its high

adsorption capacity such that it can be retained in the oral cavity for long periods, the maximum retention lasts for up to 12 h.<sup>[15]</sup> Impregnation of these agents into acrylic liners may prolong their efficiency. In our study, clotrimazole in acrylic reliner and acrylic tissue conditioner showed that concentration was maximum on 8th day and uniformly decreased thereafter. The maximum amount of release up to 8th day was 2.52, 4.23, 9.84 mg for 2%, 5% and 10% respectively. The initial high release is a surface phenomenon where the molecules at the surface are released at this early stage. The later slower diffusion is likely to be due to the diffusion of the drug from the core of the polymer by water cluster formation around the drug articles controlled by concentration dependent diffusion.<sup>[16]</sup> Even though reliner and tissue conditioner are harden after 72 days of study, during compressive force they are not get compressed properly and returned to their original state, and so this property was not studied in this study. The release of clotrimazole with time for the different type of acrylic reliner and tissue conditioner was studied and it was found to obey first order kinetics upto 56 days, the k value was found to be 0.02 days<sup>-1</sup> and the T half was 33 days. The advantages of incorporating these antimicrobial agents into denture linings for the treatment of an infection are likely to outweigh many adverse effects and thus are more effective methods of treatment.

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