AN INVITRO STUDY TO ASSESS THE ABSORPTION AND RELEASE OF CHLORHEXIDINE FROM ACRYLIC RESIN DISCS IMMERSED IN IT THEREBY SIMULATING CLINICAL TREATMENT OF ORAL CANDIDIASIS.

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In partial fulfillment of the requirement for the degree of

MASTER OF DENTAL SURGERY

(PART II BRANCH I)
(PROSTHODONTICS AND CROWN & BRIDGE)

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CERTIFICATE

This is to certify that the dissertation titled "**AN INVITRO STUDY TO ASSESS THE ABSORPTION AND RELEASE OF CHLORHEXIDINE FROM ACRYLIC RESIN DISCS IMMERSED IN IT THEREBY SIMULATING CLINICAL TREATMENT OF ORAL CANDIDIASIS**" is a bonafide record of work carried out by Dr. PREETHY CHANDRAN, during the period of 2010-2013.This dissertation is submitted in partial fulfillment, for the degree of Masters of Dental Surgery awarded by Tamil Nadu Dr. MGR Medical University, Chennai in the branch of Prosthodontics. It has not been submitted partially or fully for the award of any other degree or diploma.

GUIDED BY

Dr. C. Sabarigirinathan MDS

Professor/Senior Civil surgeon

Department of Prosthodontics

Tamil Nadu Govt. Dental College

& Hospital Chennai -03

HEAD OF THE DEPARTMENT

Dr. C. Thulasingam MDS

Department of Prosthodontics

Tamil Nadu Govt. Dental College

& Hospital Chennai -03

HEAD OF THE INSTITUTION

Prof. Dr. K.S.G.A. Nasser.MDS

Tamil Nadu Govt. Dental College & Hospital Chennai 600003

DECLARATION

I, **Dr. PREETHY CHANDRAN,** do hereby declare that the dissertation titled **"AN INVITRO STUDY TO ASSESS THE ABSORTION AND RELEASE OF CHLORHEXIDINE FROM ACRYLIC RESIN DISCS IMMERSED IN IT THEREBY SIMULATING TREATMENT OF ORAL CANDIDIASIS"** was done in the Department Of Prosthodontics, Tamil Nadu Government Dental College & Hospital, Chennai 600 003. I have utilized the facilities provided in the Government Dental College for the study in partial fulfilment of the requirements for the degree of **Master of Dental Surgery** in the speciality of **Prosthodontics and Crown & Bridge (Branch I)** during the course period **2010-2013,** under the conceptualization and guidance of my dissertation guide, **Dr. SABARIGIRINATHAN MDS.**

I declare that no part of the dissertation will be utilized for gaining financial assistance for research or other promotions without obtaining prior permission from the Tamil Nadu Government Dental College & Hospital.

I also declare that no part of this work will be published either in the print or electronic media except with those who have been actively involved in this dissertation work and I firmly affirm that the right to preserve or publish this work rests solely with the prior permission of the Principal, Tamil Nadu Government Dental College & Hospital, Chennai 600 003, but with the vested right that I shall be cited as the author(s).

Signature of the PG student **Signature of the HOD**

Signature of the Head of the Institution

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And

Dr. Preethy Chandran aged 30 years currently studying as **Post Graduate Student** in the Department of Prosthodontics, Tamil Nadu Government Dental College and Hospital, Chennai-03 (herein after referred to as the 'PG Student and co investigator').

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Witness **PG Guide**

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My salutations to ALMIGHTY – A tangent between zero and infinity – for his divine grace bestowed when needed.

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ABSTRACT

AIM & OBJECTIVES: The aim of this study is to assess the absorption and release of Chlorhexidine from acrylic heat cure and light cure resin discs simulating treatment of oral candidiasis.

MATERIALS&METHODS: 10mm discs of prepolymerised heat cure acrylic discs and light cure resin discs were immersed in Chlorhexidine solution for 10 hours, after which they are washed dried & placed in distilled water.20µl of this is subjected to High Performance Liquid Chromatography analysis for every one,three and fifth hour. After a week this is subjected to Scanning Electron Microscopy.

RESULTS: In comparison to the standard release of Chlorhexidine at 2.48 and 2.85 min the samples of heat cure showed the similar peaks but the light cure discs containing urethane dimethacrylate and triethylene dimethacrylate released much less.

CONCLUSION: The peaking of Chlorhexidine from samples suggest that the retention time and area of peak is less than that of standard but effective against denture induced stomatitis especially in patients who are immunocompromised, diabetics and on long term antibiotics.

KEY WORDS: Chlorhexidine, High Performance Lquid Chromatography, Scanning Electron Microscopy.

INTRODUCTION

Oral candidiasis is the most common infection affecting oral mucosa in complete denture wearers.[1]Candida associated denture stomatitis is characterized by generalized inflammation of palatal mucosa covered by the denture. 72% of population wearing denture are prone to develop Candida associated denture stomatitis.[2] It is considered as possible spearhead in seriously ill patients like those under antibiotics, steroids and the immunocompromised.[3]It can be disruptive to dental treatment and a barrier to patient's well being.

Candida albicans is considered the principal pathogen in the development of Candida associated denture stomatitis.[4]Microbiological examination indicates, in most cases the upper denture as the major source of infection. The ability to adhere and colonize on denture acrylics is the most important property of Candida which in turn leads to Candida associated denture stomatitis.

The combination of entrapment of yeast cells in the irregularities in denture base and denture relining materials, poor oral hygiene and several systemic factors is the most probable cause of Candida associated denture stomatitis. These factors combined together not only predispose Candida species to become pathogenic from being a commensal but also makes treatment more complex.

Despite the usage of a number of effective antimycotics for the treatment of oral candidasis, failure of therapy is not uncommon owing to the unique environment of the oral cavity where the flushing effect of saliva and the cleansing action of the oral musculature tend to reduce the drug concentration to sub-therapeutic levels[5].

The regular treatment protocol for Candida Associated Denture Stomatitis are as following

1. Control of dental plaque
2. Use of antifungals
3. Antiseptic mouth wash
4. Denture immersed in mouth rinses
5. Avoidance of denture trauma
6. Emphasis on maintaining good hygiene

The major drawback with using the mouth rinses is that only 30% is retained in mouth, 24 hours after 1 min rinse as the solution is diluted by saliva and washed away by the cleansing effect of oral musculature. So only a brief exposure of high concentration of mouth rinse is noted, after which only sub therapeutic doses stay in mouth.[6] These doses do not reach the minimum inhibitory concentration to clear candidiasis leading to their recurrence.

In order to overcome this drawbacks of mouth rinses studies were conducted to use polymerized acrylic resins as carriers for drugs orally[7]. Similarly, soft liners and tissue conditioners placed in dentures have been used as carriers for antifungal drugs in treating denture stomatitis.[8] Advantages of this method of drug delivery include no need for patient compliance, simultaneous treatment of injured denture-bearing tissues as well as that of candidal infection and reduced application frequency.

Although polymethylmethacrylate resin surfaces could represent an important predisposing factor for micro organisms colonization they have good fracture resistant,

satisfactory optical properties, esthetics, easy manipulation, reasonable cost and being inert to oral cavity. [9.]

These acrylic resins can absorb water and chemical compounds from the oral environment, and also release these components into the surrounding environment[10.] Due to the polymeric nature, it is possible that continuous exposure of polymethylmethacrylate resin surfaces to antifungal agents permits their absorption and release after treatment interruption, which could be detected as a residual effect[11].

Keeping this phenomenon in mind the study has been conducted to verify if the concentration of the chemicals are available to affect the susceptible species for an extended period until the lesion heals.

This study concentrates on usage of prepolymerised discs soaked with mouth rinses as a drug delivery system locally there by overcoming the short comings in using antifungals systemically for a extended period of time due to the tenacious capacity of candidiasis to keep recurring.

AIM

The aim of this invitro study was to assess if there was absorption and release of Chlorhexidine from the heat cure acrylic resin discs and light cure resin discs thereby simulating treatment of oral candidasis.

OBJECTIVES

1. To assess the amount of Chlorhexidine released from the heat cured resin discs from 2% Chlorhexidine solutions.
2. To assess the amount of Chlorhexidine released from light cured resin discs from 2% Chlorhexidine solutions.
3. To compare the surface details of the heat cured resin discs before and after treatment simulation.
4. To compare the surface details of the light cure resin discs before and after treatment simulation.
5. There by assessing that the immersion of these discs in Chlorhexidine allows release of the drug regularly in minimum inhibitory concentration to prevent recurrence of Candida albicans.

ADDY M, WRIGHT R.(1978)[12] et al conducted a clinical and laboratory studies to compare the antibacterial properties of two antiseptic mouthwashes, 1% povidone iodine and 0.2% Chlorhexidine gluconate. In a group of 10 subjects after a single rinse with povidone iodine, an immediate mean fall in total salivary aerobes and anaerobes occurred, followed by a return to normal levels by 1-hour postrinsing .With Chlorhexidine gluconate a similar but greater reduction in salivary bacterial counts was observed, which was still present up to the 7-h postrinsing period. The results suggest that povidone iodine, as a mouthwash, exerts only an immediate antibacterial effect and unlike Chlorhexidine , which is retained at antibacterial levels within 5the oral cavity after expectoration. This lack of prolonged action of povidone iodine in the oral cavity would appear to be relevant to its reported lack of antiplaque activity.

ADDY M, HANDLEY R.(1981)[13] et al determined the effects on the water uptake and weight loss of the incorporation of Chlorhexidine acetate in heat cured acrylic and an acrylic gel soft liner was determined. Following soaking in water for 87 days the heat cured test specimens demonstrated an initial weight gain followed by a progressive loss, the soft liner test specimens however all showed weight gains. However these weight losses were less than the Chlorhexidine content for 5 and 10% admixtures. Hardness and moduli of elasticity measurements were significantly reduced for heat and cold cured acrylics containing Chlorhexidine.. However, these do not negate the carriage of medicaments in these materials for prolonged delivery within the oral cavity, if employed as rebase or reline materials in existing prostheses.

ADDY M.(1981)[14] et al incorporated Chlorhexidine acetate at concentrations of 2, 5 and 10% into the powder phase of heat and cold cured acrylics and an acrylic gel soft liner. At 10% admixture the release from the polymerized and plasticized acrylics was in excess of 100 days. Light and scanning electron microscopy of polished and fractured surfaces of the polymerized acrylic showed the Chlorhexidine distributed throughout the matrix of the material.

ADDY M, RAWLE L, HANDLEY R, NEWMAN HN, COVENTRY JF(1982)[15] et al Conducted a study in which polyethylmethacrylic strips of suitable dimensions containing 10 to 50% Chlorhexidine acetate, 40% metronidazole and 40% tetracycline were prepared. Daily release of the incorporated drugs into 1 ml aliquots was measured spectrophotometrically over a 14 day period. The strips appear to have potential for prolonged drug delivery to periodontal pockets. Preliminary clinical use revealed no patient acceptability problems an alterations in subgingival flora were produced.

ADDY M, THAW M(1982)[16] et al investigated the release of Chlorhexidine acetate, prednisolone sodium phosphate, and prednisolone alcohol from a cold cured acrylic denture base material. Water, at the concentrations of 10%, 20%, and 30% of the monomer phase of the material was added to Chlorhexidine-containing and Chlorhexidine-free samples. Spectrophotometric and bioassay measurements of the release of Chlorhexidine demonstrated protracted delivery to 140 days.

Scanning Electron Microscopic examination of the polished, etched, and fractured surfaces of the material demonstrated that Chlorhexidine acetate and

prednisolone sodium phosphate were incorporated into the interbead matrix areas of the material.

D.J. LAMB,M.V. MARTIN (1983)[17] et al fitted chlorhexidine acetate incorporated autopolymerizing acrylic resin to palate of rats Chlorhexidine was found to diffuse out of acrylic in fungicidal concentrations for up to three weeks when mixed with the acrylic powder in the proportion of 7.5% (w/w). At this concentration it was found that palatal candidosis was cured or prevented. However, rats fitted with Chlorhexidine supplemented plates were found not to take sufficient food during the experimental period to maintain their body weight.

JANEM.C.COURTIET, W. MACFARLANAEN, AND L. P. SAMARANAYAKE (1985)[18] et al determined the effect of pretreatment of denture acrylic with Chlorhexidine gluconate on the subsequent adherence of Candida albicans gdh 2346 . Adherence was significantly reduced by pretreatment with chlorhexidine; maximal inhibition was achieved by incubation at room temperature for 30 min in 2% Chlorhexidine. Inhibition of adherence was greatest when the organisms were grown in conditions that enhanced adherence the most, i.e., growth to stationary phase in high concentrations of galactose and sucrose.

J. MCCOURTIE, T. W. MACFARLANE AND L. P. SAMARANAYAKE (1986)[19] et al the preconditioned the acrylic disks with saliva for inoculation with c. albicans. Cell adhesion was compared on disk pre-coated with 0.12% Chlorhexidine gluconate. Surface coating acrylic with Chlorhexidine effectively inhibits biofilm growth and has potential therapeutic application. **TOBGI RS, SAMARANAYAKE**

LP , MACFARLANE TW. (1987)[20] et al studied the adhesion of Candida albicans to buccal epithelial cells (BEC) exposed to Chlorhexidine gluconate both in vivo or in vitro using BEC obtained from an adult and two children. There was a significant reduction in the adherence of yeasts to BEC collected immediately after an oral rinse of Chlorhexidine.

MIRTH DB, BARTKIEWICZ A, SHERN RJ, LITTLE WA(1989)[21] et al combined copolymers of hydroxyethyl methacrylate (HEMA) and methyl methacrylate (MMA) to fabricate a membrane-controlled reservoir-type controlled-release delivery system for Chlorhexidine that should be suitable for intra-oral use. Chlorhexidine released on day 30 was biologically active, as determined by a serial dilution assay against streptococcus mutans. The extended release of biologically active Chlorhexidine at a controlled rate from this system suggests that it is worthy of further evaluation for the intra-oral therapy of Chlorhexidine-treatable oral infections in non-compliant and physically or mentally compromised individuals.

SPIECHOWICZ E, SANTARPIA RP 3RD, POLLOCK JJ, RENNER RP. (1990)[22] et al evaluated at the in vitro level the antifungal effectiveness of nystatin, Chlorhexidine, and a homologous histidine polypeptide on the surface of acrylic resin disks.. Results indicated that pretreatment with poly-L-histidine was not protective against Candida albicans adherence and growth regardless of whether disks were stored in water or in the open air for the 8-hour period following yeast contamination. Chlorhexidine was totally effective in preventing Candida albicans attachment to, and growth on, the acrylic resin, even after a period of 8 days of

turbidimetric monitoring. Pretreatment with nystatin, followed by drying, was protective, yielding results similar to those obtained with Chlorhexidine.

BUDTZ-JÖRGENSEN E.(1990)[23] et al quoted that after colonization and adhesion of Candida to the epithelial surface the subsequent mucosal lesion is due to tissue destruction by potent proteolytic enzymes or toxins and an inflammatory response to Candida antigens. In denture stomatitis colonization of the fitting denture surface by Candida should be controlled by, for example, using a Chlorhexidine solution as a denture disinfectant. However, recurrences are frequent if the local or the systemic predisposing conditions are not corrected. fluconazole, a new bis-triazole, may be important for long-term treatment of immunocompromised patients.

WILKIESON.C,SAMARANAYAKE.L.P, MACFARLANE.T.W,LAMEY P.J. **MACKENZIE D(1991)[24]** et al conducted a survey of 137 patients in long term hospital care, they were examined to determine the prevalence, nature and most important causes of oral candidosis in the hospitalized elderly. The prevalence of chronic atrophic candidosis in denture wearers was 38%, while 26% of all patients had angular cheilitis, 67% of which had an infective etiology. Microbiologic examination strongly indicated the upper denture as the major source of infection in those with dentures despite the existence of a ward policy which should have encouraged good oral and denture hygiene.

LAL (1992)[25] et al conducted a study to test the effect of peridex oral rinse in five denture stomatitis patients demonstrating Candida albicans on both maxillary dentures

and palates. The patients were instructed to use peridex oral rinse containing 0.12% Chlorhexidine gluconate for a period of 24 days. They were instructed to use peridex rinse twice daily, morning and night, as a mouth rinse by swishing 15ml for 30 to 60 seconds. In addition they were asked to soak their dentures overnight in peridex solution. The photographs of palatal mucosa and agar replicas were made at baseline i.e. pretreatment, 14 days later and final time after 5 weeks. Agar replaces of the tissue-fitting surfaces of the maxillary dentures revealed elimination of Candida albicans on 14^{th} day of treatment. There was also significant decrease of palatal inflammation on the 14^{th} day when compared to baseline. But there was increased inflammation as concentration of Candida albicans on the denture surface returned to pretreatment levels after several weeks after the termination of peridex oral rinses.

DARWAZEH AM (1994)[26] et al studied the effect of exposure to 0.2% Chlorhexidine gluconate in vitro and in vivo on in vitro adhesion of Candida albicans to buccal epithelial cells from diabetic and non-diabetic subjects and found that 0.2% Chlorhexidine gluconate showed significant reduction in Candidial adhesion to buccal epithelial cells in both diabetic and non-diabetic subjects. In addition to the known fungicidal effect of Chlorhexidine, it also reduces Candida albicans adhesion to oral mucosal cells.

ALDANA L, MARKER VA, KOLSTAD R, IACOPINO AM(1994)[27] et al compared the physical properties (flexure strength and surface hardness) of heat and light polymerized denture resins (Lucitone Pink and Triad) were evaluated after candidal colonization and candidal treatment regimens. Light-activated resins may be

the materials of choice for patients prone to denture stomatitis, as they have demonstrated less overall degradation from candidal treatment modalities.

GIULIANA G, PIZZO G, MILICI ME, MUSOTTO GC, GIANGRECO R(1997)[28] et al investigated the in vitro antifungal properties of seven commercial mouthrinses containing antimicrobial agents. These included cetylpyridinium chloride (CPC), chlorhexidine digluconate (CHX), hexetidine (HEX), sanguinarine (SNG), and triclosan (TRN). The minimum fungicidal concentration (MFC) against six species of yeasts was determined by a broth macrodilution method. However, the CPC-containing mouthrinse appeared more active than the other products ($P < 0.001$). TRN or HEX did not show a lethal effect on Candida albicans, Candida parapsilosis, or Candida guilliermondii. No kill-times were achieved with the SNG-containing mouthrinse. These results suggest that mouthrinses containing antimicrobial agents might represent an appropriate alternative to conventional antifungal drugs in the management of oral candidiasis. However, the effectiveness of antimicrobial mouthrinses as antifungal agents needs to be evaluated in further clinical trials

C.WEBB, C.J.THOMAS,M.D. P. WILLCOX.D. W. S. HARTY ANDK. W. KNOX(1998)[29] et al reviewed the methods used againt candidiasis like use of denture lining materials containing antifungals, antiseptic mouth rinses, denture soaks, removal of denture trauma and attention to denture hygiene.

ANA L.MACHANDO CUCCI (1998)[30] et al investigated the water sorption, solubility,bond strength of some commercially available auto polymerizing acrylic

resins and heat cure resins. They said the water sorption of acrylic resin is accompanied with the volumetric changes.

PIZZO (1998) [31] et al studied the antifungal activity of chlorhexidine containing mouth rinses in an in vitro study. 5 commercial mouth rinses containing chlorhexidine (CHX) were used. The Minimum Fungicidal Concentration (MFC) against six species of yeasts was determined by a broth macrodilution method. The kill-time of mouth rinses at half the concentration of the commercial formulations was determined. MFCs were achieved with each mouth rinses against all the organisms under test. However, significant differences in MFC values were found for Ebur Os in comparison with Dentosan, Corsodyl and Plak out ($p < 0.001$). Kill-times of Corsodyl and Dentosan were less than or 10 equal to 120 sec with all the species of yeasts, except Torulopsis glabrata. Significant differences were found in kill-time values between Dentosan and Broxo Din only ($p < 0.001$). These results suggested that CHX-containing mouth rinses might represent an appropriate alternative to conventional antifungal drugs in the management of oral Candidiosis. However effectiveness in preventing systemic fungal infections in immunocompromised patients requires further in vivo studies.

ANB ELLEPOLA ,LP SAMARANAYAKE(2000)[32] et al conducted a study to investigate the effect of brief exposure to 3 different sub therapeutic concentrations of Chlorhexidine gluconate(0.005%,0.0025%,0.00125%) on germ tube formation of Candida albicans. They concluded that it may modulate candida germ tube formation as well as its growth, thereby suppressing its pathogenecity invivo.

ARJUNA N.B. ELLEPOLA AND LAKSHMAN P. SAMARANAYAKE(2000)[33] et al quoted that Chlorhexidine has been used as an adjunct in the management of oral candidoses since its introduction in the 1970s. For instance, 0.2% Chlorhexidine gluconate has been successfully employed as a mouth-rinse in the treatment of Candida-associated denture stomatitis and in pseudomembranous candidosis, while 2% suspension is used as an overnight denture disinfectant.

Chlorhexidine gluconate has a bimodal action on Candida:

1. It is fungicidal even at very low concentrations.
2. It is capable of significantly suppressing candidal adhesion to both inorganic and organic substrates.

Its multifaceted anti-candidal action has prompted many clinicians to propose Chlorhexidine mouthrinse as an appropriate alternative to conventional antifungals in the management of oral candidosis.

P.D RIGGS, M BRADEN, M PATEL(2000)[34] et al conducted a study in which series of different methacrylate monomers (with either 1 or 2.5% dimethyl-p-toluidine, DMPT) was gelled with poly(ethyl methacrylate) powder (containing benzoyl peroxide) thus forming a room temperature curing system and doped with 5.625% Chlorhexidine diacetate . Nuclear magnetic resonance spectroscopy analysis showed release from the tetrahydrofurfuryl methacrylate-based samples considerably greater than that from other methacrylate monomers. But doping the polymer with Chlorhexidine hindered the polymerization, resulting in a higher level of residual monomer and low molecular weight components being leached from the polymer.

M.P. PATEL, A.T. CRUCHLEY, D.C. COLEMAN, H. SWAI, M. BRADEN, D.M. WILLIAMS(2001)[35] et al conducted a study on self curing system based on poly(ethyl methacrylate) and tetrahydrofurfuryl methacrylate (pem/thfm) used with Chlorhexidine diacetate (cx) added at levels between 0 and 12% w/w. There was an initial high release of cx over 24 h followed by a slow dilution up to 7 days. cx inhibited candidal growth and survival markedly in vitro, with the test samples showing less than 0.5_10__cfu/ml compared to controls (3}4_10__ cfu/ml). These results indicate the potential of a chlorhexidine containing pem/thfm polymeric system in the treatment of persistent candidal infections.

SILVIA-EDITH CALAMARI , MARÍA-ALEJANDRA BOJANICH , SILVINA-RUTH BAREMBAUM, NORA BERDICEVSKI, ANA-ISABEL AZCURRA;(2001)[36] et al conducted a study to assess the antifungal and post-antifungal effects of Chlorhexidine, fluconazole, chitosan and its combinations on virulence factors of Candida albicans they concluded that the short exposures to sub-inhibitory concentrations of the antifungal agents under analysis, isolated or combined, can modulate the way virulence factors get manifested, thus decreasing their pathogenicity.

FERGUSON (2002)[37] et al conducted an in vitro susceptibility test of Candida albicans to various commonly used intra canal irrigants and medication for eliminating Candida albicans from infected root canal systems. The minimum inhibitory concentration of sodium hypochlorite (NaOCl), hydrogen peroxide (H2O2), aqueous calcium hydroxide and Chlorhexidine di gluconate that is required to kill a standardized inoculum of Candida albicans were determined. Growth of the yeast was

measured by optical density. Descriptive and inferential statistics were performed on the data obtained. It was found that all the medicaments exhibited anti fungal effect except calcium hydroxide. Chlorhexidine di gluconate exhibited anti fungal effect at <0.63µg\ml. This study concluded that Naocl, H2O2, and Chlorhexidine digluconate diffuse through the root canal and will be effective fungicidal agents, even if they become significantly diluted in the process.

K.J. ANUSAVICE, N.-Z. ZHANG, AND C. SHEN (2006)[38] et al tested the hypothesis that the release of Chlorhexidine from a urethane dimethacrylate and triethylene glycol dimethacrylate resin system can be effectively controlled by the Chlorhexidine diacetate content and ph. The filler concentrations were 9.1, 23.1, or 33.3 wt%, and the filled resins were exposed to ph 4 and ph 6 acetate buffers. The results showed that fickian diffusion was the dominant release mechanism. the rates of release were significantly higher in ph 4 buffer, which was attributed to the increase of Chlorhexidine diacetate solubility at lower ph. the higher level of filler loading reduced the degree of polymerization, leading to a greater loss of organic components and higher Chlorhexidine release rates

GUNJAN DHIR, DAVID W. BERZINS, VIRENDRA B. DHURU, A. RAJ ERIATHAMBY, AND ANDREW DENTINO, (2007)[39] et al have assessed that the addition of anionic charge on denture base resins has been shown to inhibit Candida albicans adhesion and to facilitate adsorption of salivary defense molecules. Flexural strength and modulus, water sorption, solubility, and color stability tests were conducted to ensure compliance with ada specification no. 12. .Within the

limitations of this study, the physical properties of the phosphate denture base resin at 10% should be suitable for denture fabrication based on the properties assessed.

W NITTAYANANTA, **TA DEROUEN**, **P ARIRACHAKARAN**, **T LAOTHUMTHUT**, **K PANGSOMBOON**, **SPETSANTAD**, **V VUDDHAKUL**, **H SRIPLUNG**, **SJARURATANASIRIKUL AND MD MARTIN(2008)**[40] et al conducted a study To determine if Chlorhexidine can be used as an intervention to prolong the time to relapse of oral candidiasis. A double-blinded randomized clinical trial was performed in 75 HIV/AIDS subjects with oral candidiasis. Clotrimazole troche was prescribed, and the subjects were re-examined every 2 weeks until the lesions were completely eradicated. The subjects were then randomly divided into two groups; 0.12% Chlorhexidine (*n* = 37, aged 22–52 years, mean 34years) and 0.9% normal saline (*n* = 38, aged 22–55 years, mean 38 years). They were re-examined every 2 weeks until the next episode was observed. The time to recurrence of oral candidiasis between the Chlorhexidine and the saline group was not statistically significant ($P > 0.05$). The following variables were significantly associated with the time of recurrence; frequency of antifungal therapy ($P = 0.011$), total lymphocyte ($P = 0.017$), alcohol consumption ($P = 0.043$), and candidiasis on gingiva ($P = 0.048$).The subjects with lower lymphocyte showed shorter oral candidiasis-free periods ($P = 0.034$).Chlorhexidine showed a small but not statistically significant effect in maintenance of oral candidiasis-free period. This lack of significance may be due to the small sample size. Further study should be performed to better assess the size of the effect, or to confirm our findings.

TRABOULSI RS, MUKHERJEE PK, GHANNOUM MA(2008)[41] et al compared the use of inexpensive topical alternatives, e.g. oil of melaleuca (tea tree oil (TTO)), Chlorhexidine (CHX), povidone iodine (PI) and gentian violet (GV), to treat oral candidiasis in human immunodeficiency virus (HIV)-infected patients in resource-poor countries, GV showed the most potent activity against all Candida isolates tested . CHX was 64 times less active than GV. The lowest antifungal activity was seen for PI.

SPENCER REDDING,BAKUL BHATTH,RALPH RAWLSGREGG SIEGELK EVIN SCOTT, JOSE LOPEZ-RIBOT(2009)[42] et al applied several thin-film polymer formulations, with and without antifungals, to inhibit Candida albicans biofilm growth on denture material. Reduced biofilm formation between 70% and 80% with nystatin, and between 50% and 60% with amphotericin B, Chlorhexidine gave up to 98% was significantly greater than all other formulations .

WALA M. AMIN, MUNA H. AL-ALI, NESREEN A. SALIM, AND SANDRA K. AL-TARAWNEH (2009)[43] et al showed in their study that fluconazole, Chlorhexidine and the combination of the two drugs can be successfully incorporated it leached steadily out of the PMMA resin into distilled water at mouth temperature and that sustained drug release continued throughout the 28 days test period. It was also shown that the released drugs demonstrated an antifungal activity against the resistant Candida albicans and this was most remarkable in the combined drugs samples.The findings of this investigation have a clinical value in terms of their significant contribution to the treatment of fungal infections of the oral cavity.

They quoted that HPLC is used for a wide range of applications and offers significant advantages in the analysis of pharmaceutical formulations and biological fluids. An added advantage is that many detectors used in HPLC are non-destructive, thus facilitating sample recovery

CHRISTOPHER R. PUSATERIA, EDWARD A. MONACOA, AND MIRA EDGERTON (2009)[44] et al conducted a study in which acrylic disks were preconditioned with 500 µl saliva for 30 min, and inoculated with C. albicans cells (106 cells/ml) for 1 h, at 37 °C. Candidal assays were performed on 48-hour-biofilms.Cell adhesion was compared on disks pre-coated with 0.12% Chlorhexidine gluconate,. Surface coating acrylic with Chlorhexidine or Hst 5 effectively inhibits biofilm growth and has potential therapeutic application[38]

Z. CAO1 X. SUN C.-K. YEH Y. SUN (2010)[45] et al conducted a research in which polymethacrylic acid (pmaa) was covalently bound onto diurethane dimethacrylate denture resins in the curing step. The pmaa resins bound cationic antifungal drugs such as miconazole and Chlorhexidine digluconate (cg) through ionic interactions. The anticandidal activities of the drug-containing pmaa-resin discs were sustained for a prolonged period of time (weeks and months). Drugs bound to the denture materials could be "washed out" by treatment with EDTA, and the drug-depleted resins could be recharged with the same or a different class of anticandidal drugs. These results suggest clinical potential of the newly developed antifungal denture materials in the management of candida associated denture stomatitis and other infectious conditions.

SHEN, N.Z. ZHANG AND K.J. ANUSAVICE (2010)[46] et al investigated resins containing 23 wt% of filler, and the various ratios of calcium fluoride to Chlorhexidine diacetate were investigated. The release was conducted in ph 4, 5, and 6 acetate buffers. The results showed that release of either agent increased as the ph of the medium decreased. The presence of fluoride salt substantially reduced the Chlorhexidine release, while the presence of a specific quantity of Chlorhexidine significantly increased fluoride release. This interaction can be utilized to optimize the release of either agent for therapeutic purposes[40].

CARMEN SALERNO, MICHELANGELO PASCALE, MARÍA CONTALDO, VINCENZO ESPOSITO, MAURIZIO BUSCIOLANO ,LUCIO MILILLO, AGOSTINO GUIDA , MASSIMO PETRUZZI , ROSARIO SERPICO(2011)[47] et al stated Candida-associated denture stomatitis remains the more frequent form of oral candidiasis with preferential localization to the palatal mucosa. Among the predisposing local factors the main one is the accumulation of microbial plaque on the surface of the denture in contact with the mucosa.Candida-associated denture stomatitis, even if asymptomatic, should be treated as it may act as reservoir for infections more extensive and encourage the resorption of the alveolar bone.The more effective treatment is the eradication and control of the microbial plaque.

DARWISH RM, AMIN WM, AL-ALI MH, SALEM NA(2011)[48] et al Conducted a study to monitor the release of an antifungal drug, fluconazole, from a self-polymerizing poly methyl methacrylate denture base resin in artificial saliva and comparing it with the release in water. A high-performance liquid chromatography-ultra-violet (HPLC-UV) method and agar diffusion method was used. The findings

suggest that the drug leaches steadily out of the polymethylacrylate resin in artificial saliva and distilled water at mouth temperature and that sustained drug release continued throughout the 28 days test period. It was shown that the released drug demonstrated antifungal activity against both standard and resistant Candida albicans. The findings of this investigation have a clinical value in terms of their significant contribution to the treatment of fungal infections of the oral cavity.

INGRID MACHADO DE ANDRADE,, PATRICIA C. CRUZ, ,CLA´UDIA H. SILVA-LOVATO, RAPHAEL F. DE SOUZA, , MARIA CRISTINA MONTEIRO SOUZA-GUGELMIN, ,& HELENA DE FREITAS OLIVEIRA PARANHOS, DDS, PHD(2011)[49] et al conducted a trial with sixty complete denture wearers for 21 days after receiving brushing instructions. They were distributed into three groups, daily overnight soaking in water , daily immersion at home in 0.12% Chlorhexidine for 20 minutes after dinner; and a single immersion in 2.0% Chlorhexidine for 5 minutes. Biofilm coverage area was quantified on the internal surface of maxillary dentures at baseline and after 21days.Both Chlorhexidine-based treatments had a similar ability to remove denture biofilm. Immersion in 0.12% or 2.0% Chlorhexidine solutions can be used as an auxiliary method for cleaning complete dentures.

SOUKAINA RYALAT, RULA DARWISH, AND WALA AMIN(2011)[50] et al prepared two groups of disc-shaped polymethylmethacrylate specimens; samples for the first group were impregnated with Chlorhexidine powder 10% w/w and samples for the second group (controls) were not. Chlorhexidine 10%, contained in the disc

specimen demonstrated a high initial rate of elution from the polymethylmethacrylate drug-release reservoir during the first 2–7 days, followed by a controlled sustained elution process that continued throughout the 28-day test period. Chlorhexidine continued to demonstrate antifungal potential throughout the 28-day test period in the well diffusion test.

HENRIQUE MONTAGNER, FRANCISCO MONTAGNER, KATIA OLMEDO BRAUN, PAULO EDELVAR CORREA PERES BRENDA PAULA FIGUEIREDO DE ALMEIDA GOMES (2011)[51] et al evaluated the antifungal action of different agents over microwave-cured acrylic resin without polishing specimens previously contaminated with Candida albicans. : sixty specimens were immersed in bhi broth previously inoculated with the yeast and stored for 3 h at 37°c. They were divided into 5 experimental groups 2%chlorhexidine solution, 0.5% sodium hypochlorite, modified sodium hypochlorite ,effervescent agent , hydrogen peroxide .This in vitro study suggested that sodium hypochlorite-based substances and hydrogen peroxide are more efficient disinfectants against Candida albicans than 2% Chlorhexidine solution and the effervescent agent.

WANDER JOSE DA SILVA,LETICIA MACHADO GONCALVES,FERNANDA FOAT,LUIZ ROBERTO PIMENTEL TREVIZAN,ALTAIR ANTONINHA DEL BEL CURY.(2011)[11] et al conducted a study in which discs were immersed in nystatin and fluconazole and deionized water. After treatment simulation, discs were immersed in to distilled water during 3,7,10 and 14 days. High performance liquid chromatography and scanning electron

microscopy analysis was done. Within the limitations of this study it was concluded that polymethylmethacrylate resin had no drug absorption with posterior release.

DAVID W. WILLIAMS, TOMOARI KURIYAMA, SONIA SILVA, SLADJANA MALIC & MICHAEL A. O. LEWIS(2011)[52] et al quote Chlorhexidine is a cationic chlorophenyl bisbiguanide and is perhaps the most frequently used mouthwash.It binds to negatively charged Candida surfaces, and induces a loss of structural integrity, decreases adherence capability and disrupts the cell wall. Chlorhexidine's anti-candidal properties are also retained against Candida that is adhered to acrylicsurfaces and is therefore of value in the treatment of chronic erythematous candidosis. Studies have shown that 0.2% chlorhexidine gluconate mouth rinses exhibit clinical benefit in the treatment of acute erythematous and pseudomembranous candidosis.

M.M. BERTOLINI, M. PORTELA, A. CURVELO, J.F. CAVALCANTI, T.V. ROMANOS, R.M. SOARES, E. LOURENCO, AND D. TELLES(2012)[53] et al conducted a study to compare the resin discs coesoft® and trusoft®, containing 0.5, 1.0 and 2.0 wt.% of Chlorhexidine. Spectral measurements were made to follow change in optical densities of storage solution, after each 48 hours during, 40 days, changing the storage solution to evaluate the cytotoxicity in fibroblastic l929 cells, the neutral red dye-uptake technique was used, until the 28th day. For antifungal activity on Candida albicans (atcc 10231), the biofilm was allowed to develop over the resin discs surface and the bioactivity of the biofilms was measured. They concluded that chlorhexidine had a dose-related inhibitory effect on the growth of Candida albicans and was also released from both resins-based denture soft lining materials. The cytotoxcity probably do not exceed the clinically tolerable level.

MATERIALS USED FOR THIS STUDY

S.NO	MATERIAL	COMMERCIAL NAME	MANUFACTURER
1.	Heat cure resin prepolymerised discs.	Pyrex heat cure resin	Pyrex polymers india
2.	Light cure resin	Heliomolar flow	Ivoclar vivadent
3.	Chlorhexidine gluconate 2%	Asep RC	Stedman pharmaceuticals Alathur,Thiruporur Tamilnadu
4.	Polyvinylsiloxane putty material	Photosil	Dental products of India mumbhai

ARMAMTERIUM

1. Compass
2. Lead pencil
3. Measuring scale
4. Micromotor with handpiece
5. Sand paper with different grits.
6. Pumice .

METHODOLOGY

1. PREPARATION OF LIGHT CURE DISCS.
2. TREATMENT SIMULATION.
3. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY.
4. PREPARATION OF STOCK STANDARD SOLUTION AND WORKING STANDARD SOLUTION.
5. PREPARATION OF STORAGE SOLUTION
6. SCANNING ELECTRON MICROSCOPY.

1.PREPARATION OF LIGHT DISCS

Specimens measuring 40mm diameter discs were obtained (thanks to pyrex polymers) were prepared by manufacturer themselves and delivered following standard protocols. Next step is to cut these 40mm discs into smaller discs of 10mm .Using a geometric compass the 10mm circles were drawn on the discs .Diamond coated abrasive disc trimmer on a micromotor handpiece was used to cut these along the drawn circles. Thus discs of 10mm were obtained for the study.

Now using these discs, mould spaces were created in polyvinylsiloxane impression material(dental products of india).In a rectangular plastic container base and catalyst were mixed as per manufacturers instructions and pressed in. On to which the discs were placed gently without pressure after applying Vaseline before die stone is completely set. Once the polyvinylsiloxane was completely set the discs were removed thus providing space for preparing light cure discs.

On the mould space mylar strips are placed. Lightcure resin (heliomolar flow ivoclar vivadent schaan, Liechtenstein) is gently filled in the mould space without incorporating any air bubble and cured layer by layer using light cure unit. Now these light cured discs are removed using the sharp excavator. These are then polished using sandpaper starting from 320 to 800 fine grit which was then polished.

2.TREATMENT SIMULATION:

Specimens were randomly assigned into four groups , each group containing five heat cure discs as group A and light cure discs as group B in the chlorhexidine 2%.Specimens were individually immersed in test tubes containing 2ml of chlorhexidine of 2% concentration which from now on will be referred as immersion solutions.

As it has to simulate the clinical condition, after soaking over night for ten hours the discs are removed washed, dried and placed in 2ml of distilled water in test tubes each separately.The immersion solutions are changed every day for a week. The samples of the storage solution were taken on first,third and seventh day after which they are subjected to Scanning Electron Microscopy.

3.HIGH PERFORMANCE LIQUID CHROMATOGRAPHY:

The analysis was carried out on an isocratic HPLC – apparatus which consists of the following parts: HPLC Schimazu model LC 20 AT; HPLC injector type Rheodyne 7125 (USA); HPLC-UV-Vis detector type GBC (Australia), model SPD 20A. The HPLC apparatus was operated under the following working conditions:

Eluent: acetonitril / 0.01M phosphate buffer (25:75%); Eluent flow rate: 1.0 ml/min; Injection volume: 20 µl; Column: BDS – C18 (25 cm x 4.6 mm, particle size 5 µm); Detector : UV – VIS Spectrophotometer (λ : 210 nm, Range: 1.0); integrator chart speed: 0.5 cm / min, attenuation: 8

4.PREPARATION OF STOCK STANDARD AND WORKING STANDRAD SOLUTION:

Stock solution: 1 mg/mL chlorhexidine in methanol diluent.

Working solution: A 100 µL aliquot of the stock was diluted to 0.1 mg/mL using 900 µL

50% solvent A/ 50% solvent B mixture diluentA 1000 µg/ml stock standard solution was prepared for each drug by dissolving 10 mg of the drug in 10.0 ml HPLC-water. These prepared solutions were kept in a refrigerator. (Solvents: A: DI H2O/ 0.1% formic acid

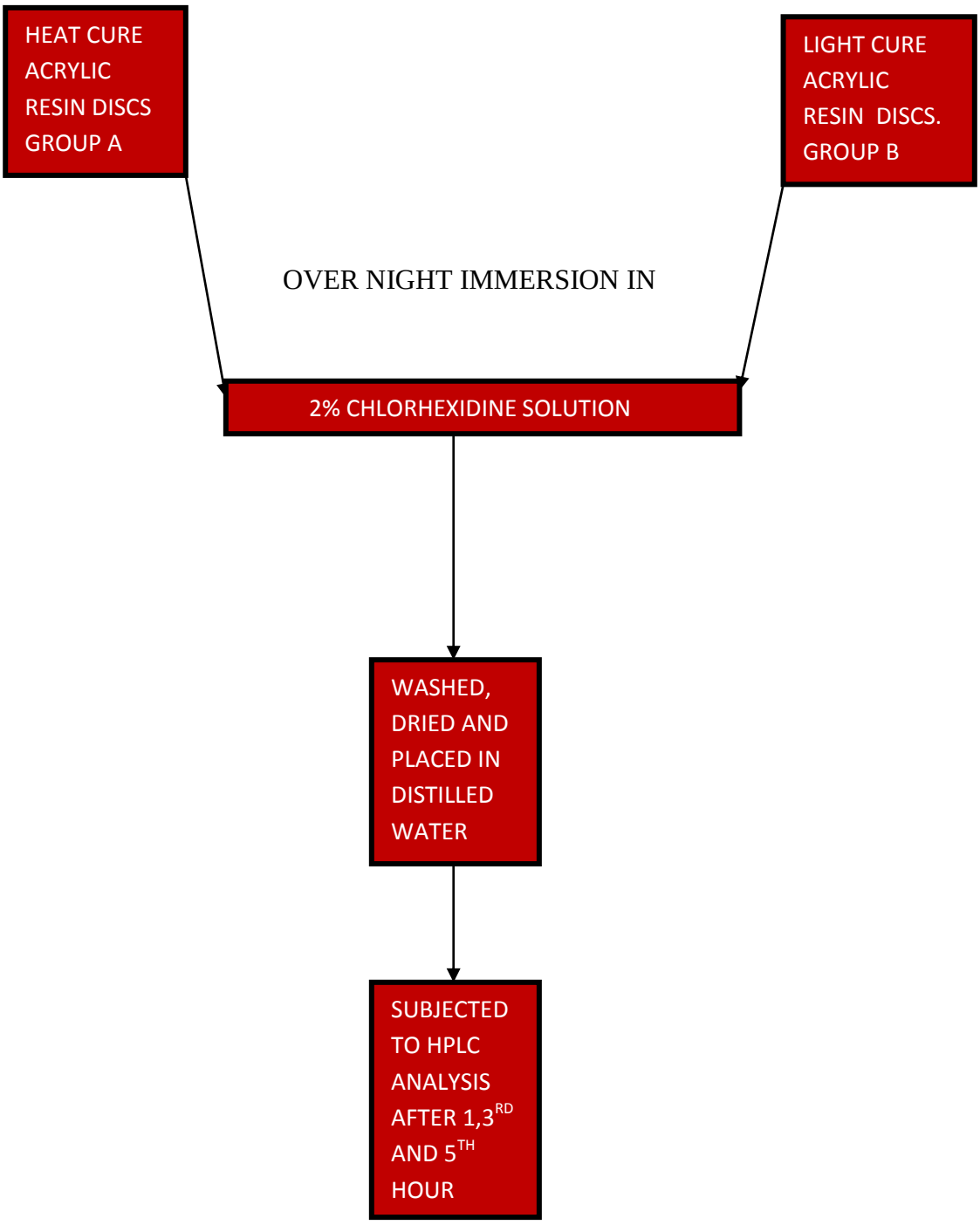
B: 97% Acetonitrile/ 3% DI H2O/ 0.1% formic acid).

5.PREPARATION OF STORAGE SOLUTION:

After 1 hour, 150 µL of aqueous solution of the corresponding internal standard (160 nmol/mL) was added and mixed. Finally, 20 µL of this mixture were injected onto the high-performance liquid chromatography column according to the below mentioned conditions. This procedure was repeated at three hours and five hours. The same procedure was repeated for the five light cure discs that is the group B specimens from which samples are taken after one hour, third hour and fifth hour. Immersion solutions were changed daily.The storage solution was subjected to HPLC analysis after one hour, third hour fifth hour.

6.SCANNING ELECTRON MICROSCOPY:

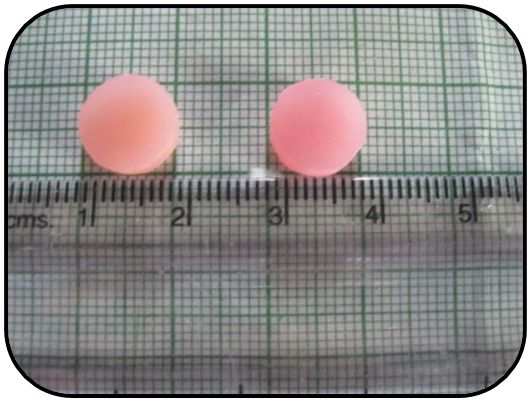
In order to determine if there was deposition of drugs on the acrylic resin surface , before and after treatment specimens were visualized with SEM magnifications starting from 50,200,500,750 and 1000.


HEAT CURE ACRYLIC RESIN DISCS GROUP A
LIGHT CURE ACRYLIC RESIN DISCS. GROUP B
OVER NIGHT IMMERSION IN
2% CHLORHEXIDINE SOLUTION
WASHED, DRIED AND PLACED IN DISTILLED WATER
SUBJECTED TO HPLC ANALYSIS AFTER 1,3RD AND 5TH HOUR

HEAT CURE ACRYLIC

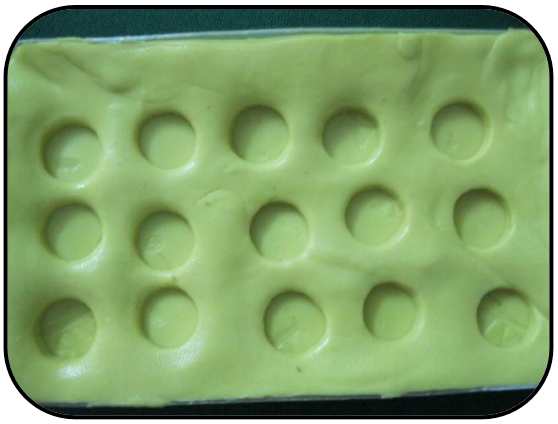
10MM CIRCLES DRAWN WITH COMPASS

ARMAMENTARIUM FOR CUTTING DISCS

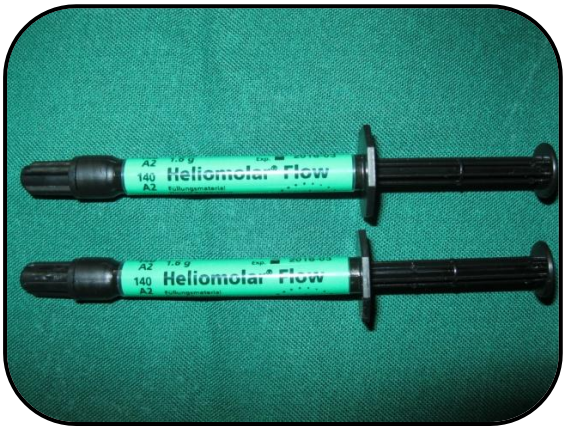


10MM DISCS

10MM DISCS IN PUTTY



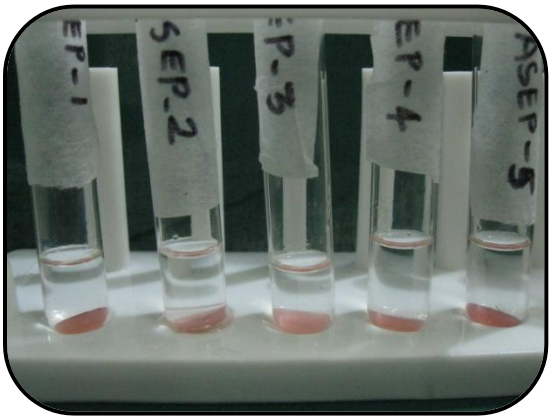
MOULD SPACE CREATED BY HEAT



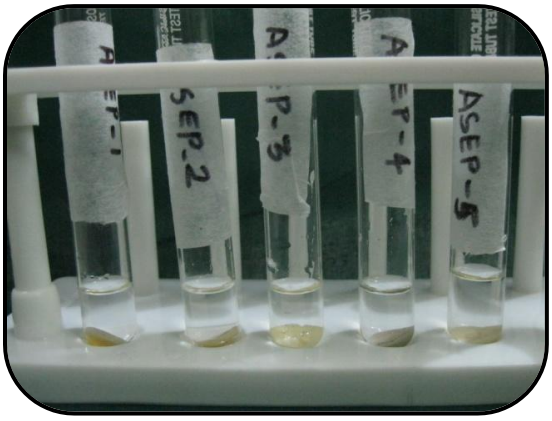
HELIOMOLAR LIGHT CURE FLOW

LIGHT CURE DISCS

ASEP RC CHLORHEXIDINE GLUCONATE SOLUTION

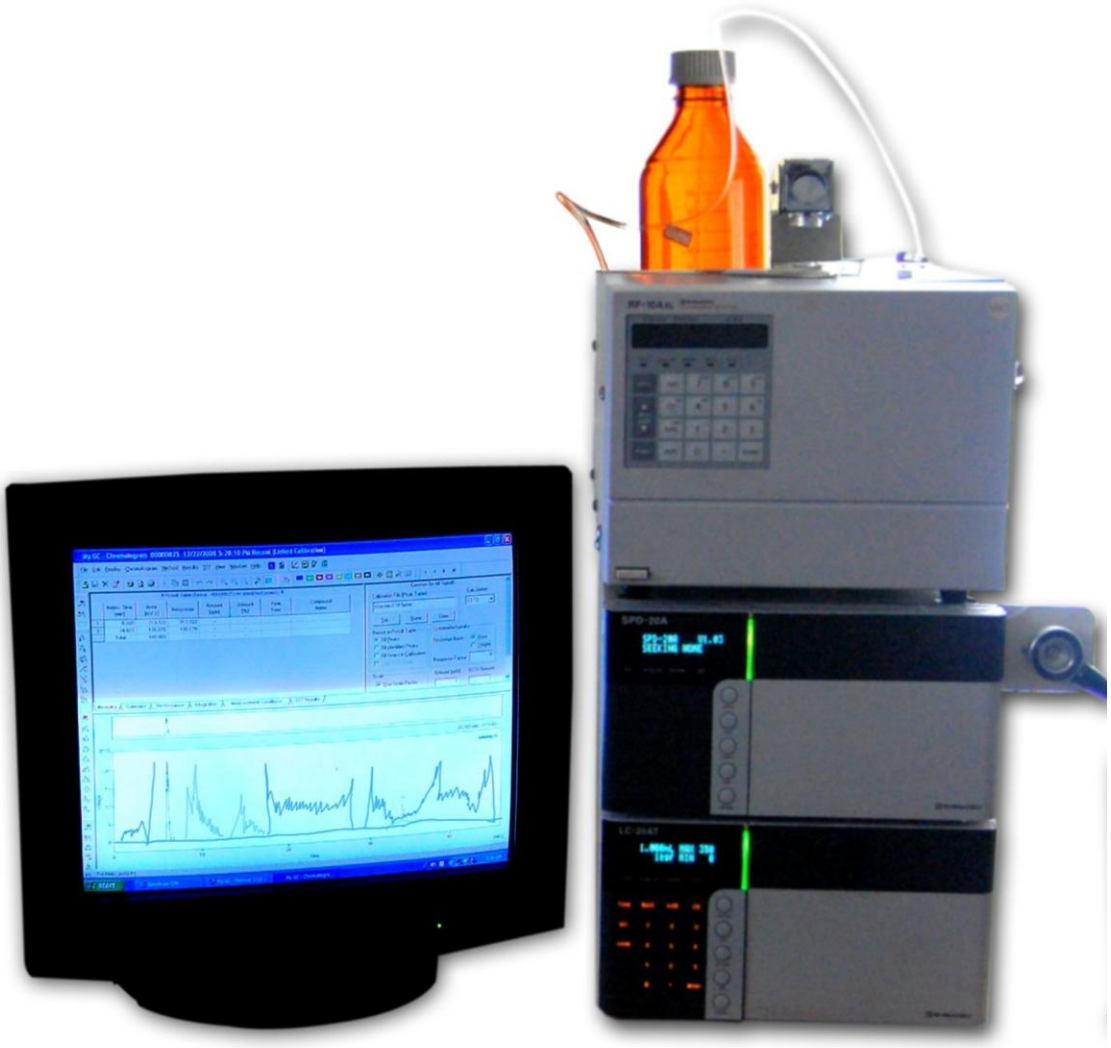


HEAT CURE DISCS IMMERSED INASEP



LIGHT CURE DISCS IMMERSED IN ASEP

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

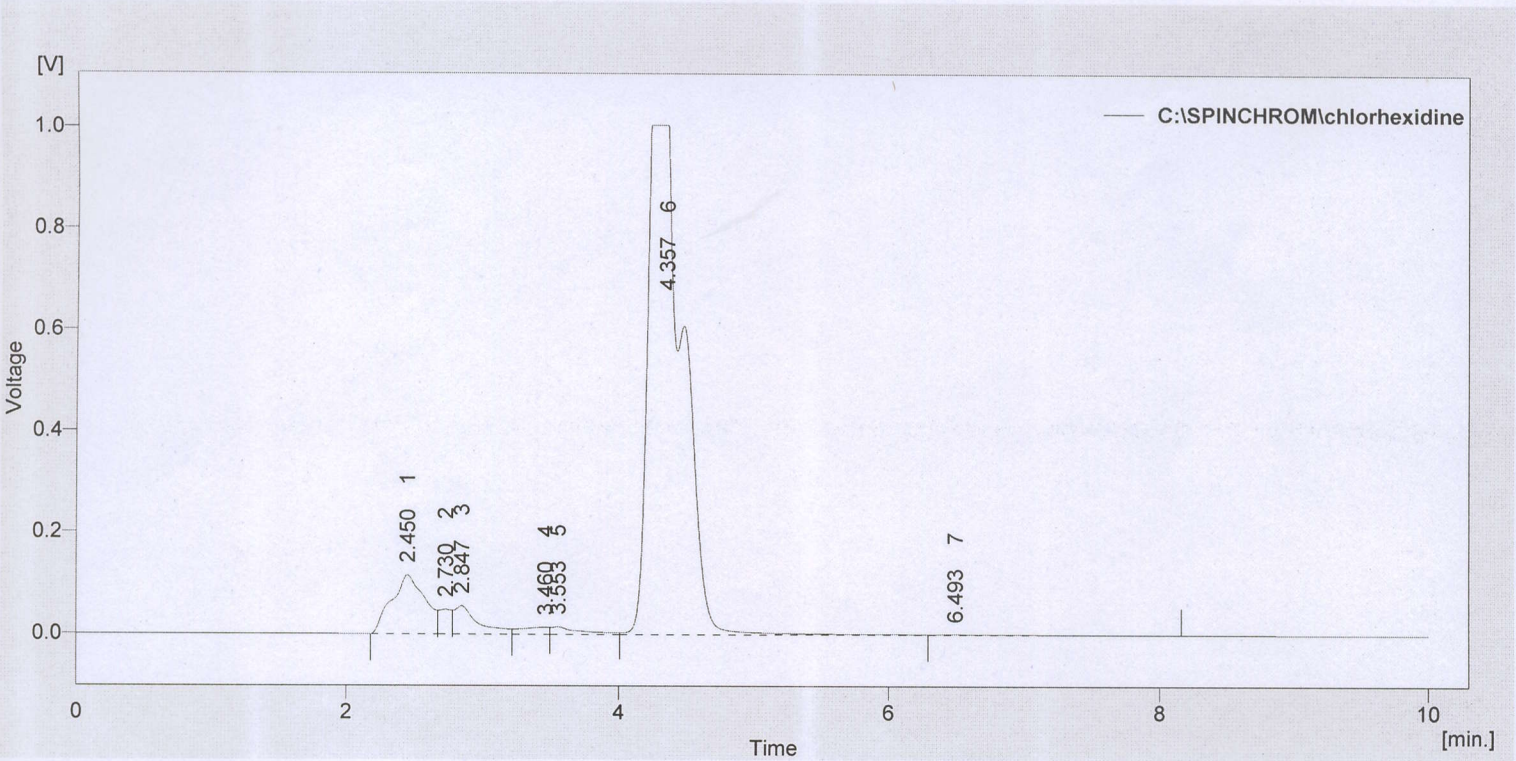


Tamilnadu Veterinary & Animal Sciences University

Dept PLAFFS- Shimadzu HPLC Report

Sample Info:

Sample ID	: chlorhexidine	Amount	: 20
Sample	: Std	ISTD Amount	: 0
Inj. Volume [ml]	: 20	Dilution	: 1
Method	: Noname	By	: Administrator
Description	: chlorhexidine		
Created	: 2/12/2011 2:29 PM	Modified	: 1/1/2001 3:16 AM
Column	: C18	Detection	: UV (Shimadzu- Promience- SPD-20A)
Mobile Phase	: acetonitrile:buffer(25:75)	Temperature	: Ambient
Flow Rate	: 1ml/minute	Pressure	: 100 kgf/cm2
Note	:		
Autostop	: 10.00, min	External Start	: Start - Restart, Down
Detector 1	: Signal 1	Range 1	: Bipolar, 1250 mV, 10 Samp. per Sec.
Subtraction chromatogram	: (None)	Matching	: No Change



Result Table (Uncal - C:\SPINCHROM\chlorhexidine)

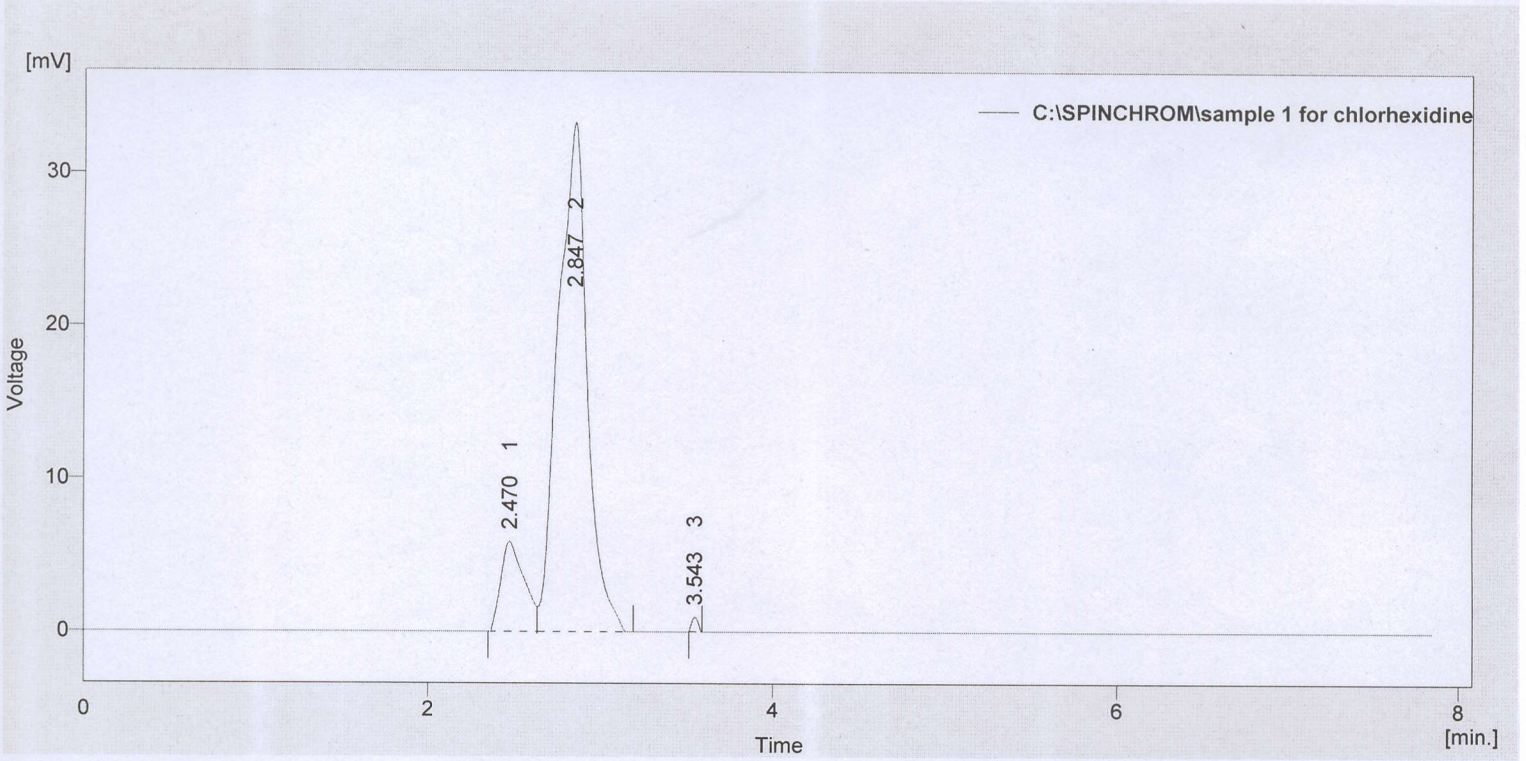
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.450	1963.392	116.217	8.9	9.2	0.31
2	2.730	304.539	48.599	1.4	3.9	0.11
3	2.847	687.729	55.850	3.1	4.4	0.16
4	3.460	202.847	13.959	0.9	1.1	0.28
5	3.553	226.527	14.938	1.0	1.2	0.18
6	4.357	18626.044	1005.710	84.4	80.0	0.33
7	6.493	64.736	1.428	0.3	0.1	0.68
	Total	22075.814	1256.699	100.0	100.0	

Tamilnadu Veterinary & Animal Sciences University

Dept PLAFFS- Shimadzu HPLC Report

Sample Info:

Sample ID	: Asep-RC	Amount	: 20
Sample	: light cure	ISTD Amount	: 0
Inj. Volume [ml]	: 20	Dilution	: 1
Method	: C:\SPINCHROM\WORK1\DATA	By	: Administrator
Description	: chlorhexidine		
Created	: 2/12/2011 2:29 PM	Modified	: 1/1/2001 3:35 AM
Column	: C18	Detection	: UV (Shimadzu- Promience- SPD-20A)
Mobile Phase	: acetonitrile:buffer(25:75)	Temperature	: Ambient
Flow Rate	: 1ml/minute	Pressure	: 100 kgf/cm2
Note	:		
Autostop	: 7.00, min	External Start	: Start - Restart, Down
Detector 1	: Signal 1	Range 1	: Bipolar, 1250 mV, 10 Samp. per Sec.
Subtraction chromatogram	: (None)	Matching	: No Change



Result Table (Uncal - C:\SPINCHROM\sample 1 for chlorhexidine)

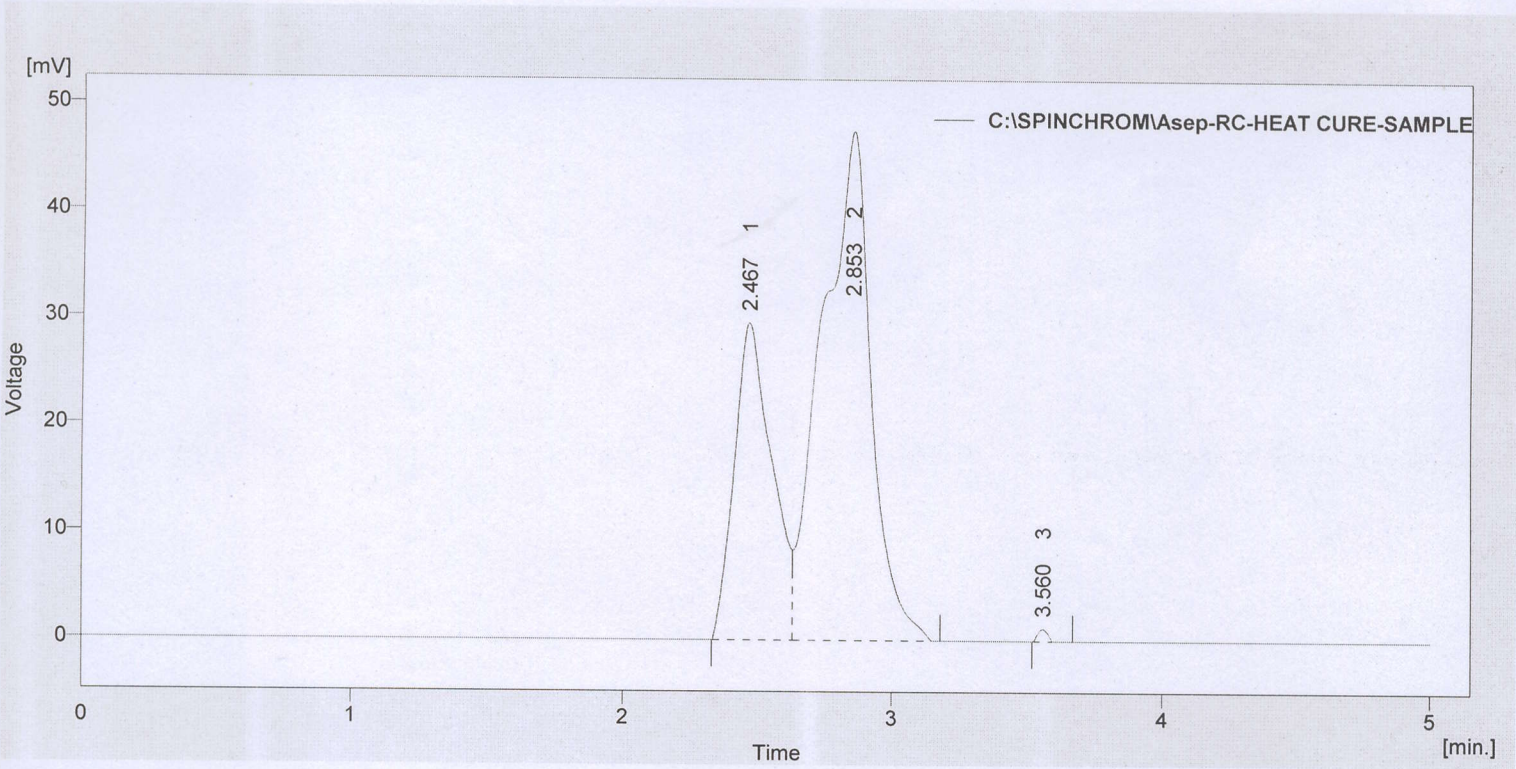
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.470	55.088	5.886	12.3	14.6	0.16
2	2.847	390.486	33.368	87.1	83.0	0.19
3	3.543	2.519	0.953	0.6	2.4	0.05
	Total	448.093	40.207	100.0	100.0	

Tamilnadu Veterinary & Animal Sciences University

Dept PLAFFS- Shimadzu HPLC Report

Sample Info:

Sample ID	: Chlorhexidine	Amount	: 20
Sample	: Asep-RC-Heat cure	ISTD Amount	: 0
Inj. Volume [ml]	: 20	Dilution	: 1
Method	: C:\SPINCHROM\WORK1\DATA	By	: Administrator
Description	: Chlorhexidine		
Created	: 2/12/2011 2:29 PM	Modified	: 1/1/2001 3:44 AM
Column	: C18	Detection	: UV (Shimadzu- Promience- SPD-20A)
Mobile Phase	: acetonitrile:buffer(25:75)	Temperature	: Ambient
Flow Rate	: 1ml/minute	Pressure	: 100 kgf/cm2
Note	:		
Autostop	: 5.00, min	External Start	: Start - Restart, Down
Detector 1	: Signal 1	Range 1	: Bipolar, 1250 mV, 10 Samp. per Sec.
Subtraction chromatogram	: (None)	Matching	: No Change



Result Table (Uncal - C:\SPINCHROM\Asep-RC-HEAT CURE-SAMPLE)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.467	287.301	29.629	32.0	37.8	0.16
2	2.853	606.892	47.601	67.6	60.7	0.22
3	3.560	2.987	1.155	0.3	1.5	0.05
	Total	897.180	78.385	100.0	100.0	

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY RESULTS

TAB NO 1 HEAT CURE DAY 1 ONE HOUR SAMPLE

PEAK 1				PEAK 2			PEAK 3		
Sample No	RT (min)	Area mV/s	Height mV	RT (min)	Area mV/s	Height mV	RT (min)	Area mV/s	Height mV
1	2.46	287.30	29.60	2.85	606.80	47.60	3.56	2.98	1.15
2	2.47	287.40	29.80	2.83	607.20	47.80	3.58	3.04	1.10
3	2.45	287.28	29.50	2.86	606.70	47.50	3.51	2.96	1.18
4	2.47	287.34	29.40	2.84	606.88	47.48	3.55	3.00	1.18
5	2.46	287.26	29.60	2.85	606.90	47.60	3.55	2.90	1.20

TAB NO 2 LIGHT CURE DAY 1 ONE HOUR SAMPLE

PEAK 1				PEAK 2			PEAK 3		
Sample No	RT (min)	Area mV/s	Height mV	RT (min)	Area mV/s	Height mV	RT (min)	Area mV/s	Height mV
1	2.47	55.08	5.88	2.84	390.48	33.36	3.54	2.51	0.95
2	2.48	55.00	5.92	2.82	391.04	33.40	3.56	2.53	0.92
3	2.47	55.28	5.84	2.80	391.28	33.16	3.52	2.48	0.98
4	2.46	54.98	5.94	2.88	389.48	32.98	3.50	2.52	0.96
5	2.46	55.14	5.88	2.84	392.14	33.56	3.54	2.48	0.94

TAB NO 3 HEAT CURE DAY 3 ONE HOUR SAMPLE

	PEAK 1			PEAK 2			PEAK 3		
Sample No	RT (min)	Area mV/s	Height mV	RT (min)	Area mV/s	Height mV	RT (min)	Area mV/s	Height mV
1	2.48	287.4	29.8	2.8	607.1	47.9	3.55	2.92	1.22
2	2.46	287.34	29.6	2.82	606.2	47.4	3.48	2.88	1.18
3	2.47	287.42	30	2.84	606.37	47.5	3.52	3.02	1.13
4	2.46	287.52	29.7	2.81	607.31	47.18	3.59	2.98	1.24
5	2.45	287.28	30.1	2.84	606.87	47.56	3.5	3.08	1.1

TAB NO 4 LIGHT CURE DAY 3 ONE HOUR SAMPLE

	PEAK 1			PEAK 2			PEAK 3		
Sample No	RT (min)	Area mV/s	Height mV	RT (min)	Area mV/s	Height mV	RT (min)	Area mV/s	Height mV
1	2.4	55.3	5.82	2.82	390.4	33.56	3.56	2.54	0.88
2	2.48	55.04	5.98	2.88	391.8	33.4	3.48	2.48	0.92
3	2.5	54.88	5.92	2.9	392.2	33.18	3.6	2.52	0.98
4	2.38	55.26	5.8	2.82	390	32.88	3.46	2.44	0.86
5	2.48	55.38	5.94	2.86	392.4	33.36	3.42	2.57	0.94

TAB NO 5 HEAT CURE DAY 7 ONE HOUR SAMPLE

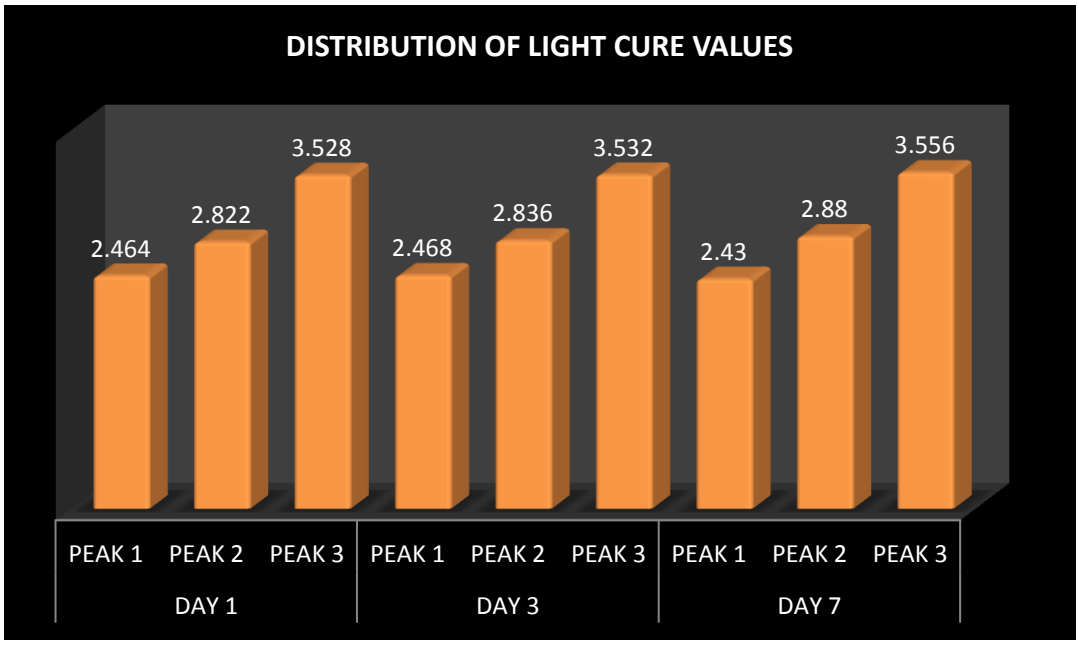
	PEAK 1			PEAK 2			PEAK 3		
Sample No	RT (min)	Area mV/s	Height mV	RT (min)	Area mV/s	Height mV	RT (min)	Area mV/s	Height mV
1	2.48	287.48	29.4	2.88	606.69	47.3	3.45	2.94	1.3
2	2.43	287.38	29.5	2.86	606.5	47.8	3.59	2.96	1.16
3	2.45	287.3	29.8	2.87	607.23	47.9	3.53	3.02	1.2
4	2.47	287.5	29.7	2.83	606.35	47.76	3.51	3.04	1.18
5	2.44	287.2	29.9	2.88	606.77	47.56	3.57	2.98	1.16

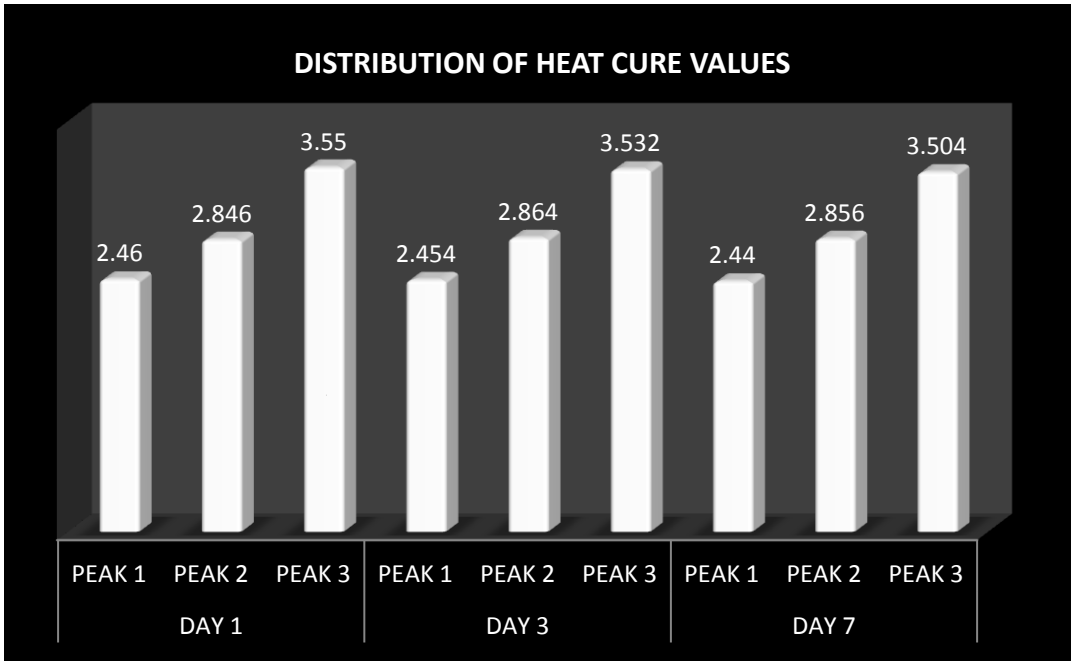
TAB NO 6 LIGHT CURE DAY 7 ONE HOUR SAMPLE

	PEAK 1			PEAK 2			PEAK 3		
Sample No	RT (min)	Area mV/s	Height mV	RT (min)	Area mV/s	Height mV	RT (min)	Area mV/s	Height mV
1	2.45	55.08	5.84	2.84	391.4	34.12	3.62	2.51	0.96
2	2.39	54.96	5.88	2.92	390.04	32.16	3.56	2.48	0.92
3	2.47	55.12	5.9	2.84	390.28	33.24	3.58	2.5	0.88
4	2.45	55.18	5.78	2.88	389.98	33.18	3.5	2.48	0.86
5	2.39	54.98	5.86	2.92	392.4	34.14	3.52	2.56	0.96

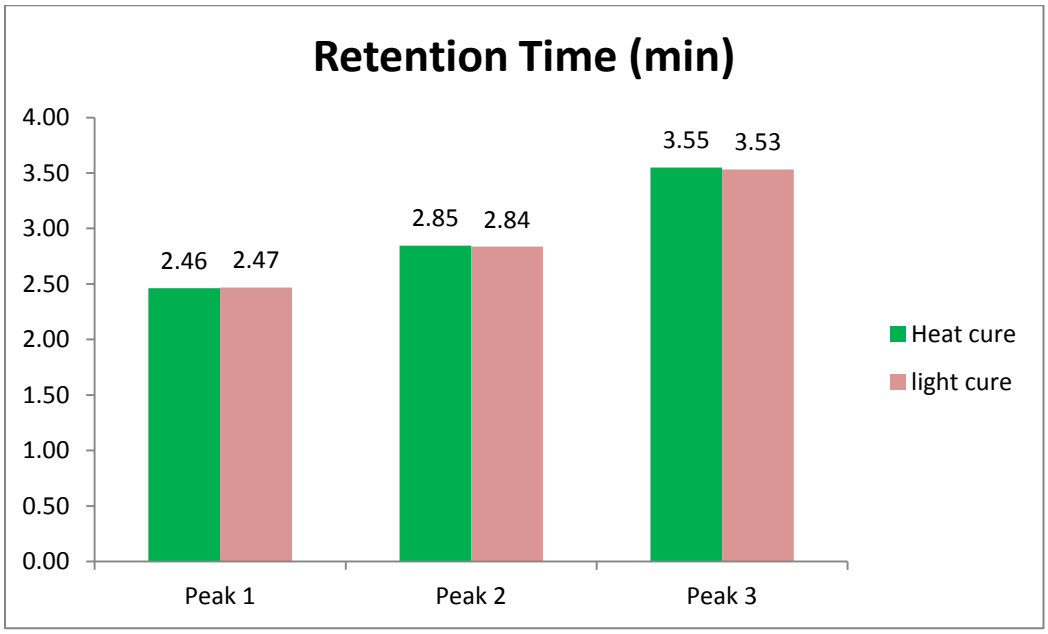
COMPARISON WITH THE STANDARD VALUES IN HEAT CURE OF RT					
		MEAN	**S.D**	**t-VALUE**	**P-VALUE**
DAY 1	**PEAK 1**	2.46	0.008	3.207	**0.033***
	PEAK 2	2.846	0.0114	-0.196	0.854
	PEAK 3	3.55	0.0255	-0.263	0.805
DAY 3	**PEAK 1**	2.454	0.0207	0.431	0.688
	PEAK 2	2.864	0.0207	1.833	0.141
	PEAK 3	3.532	0.0547	-0.939	0.401
DAY 7	**PEAK 1**	2.44	0.054	-0.083	0.938
	PEAK 2	2.856	0.0357	0.562	0.06
	PEAK 3	3.504	0.074	-1.48	0.213
*** SIGNIFICANTLY DIFFERENT WITH STANDARD VALUE**					

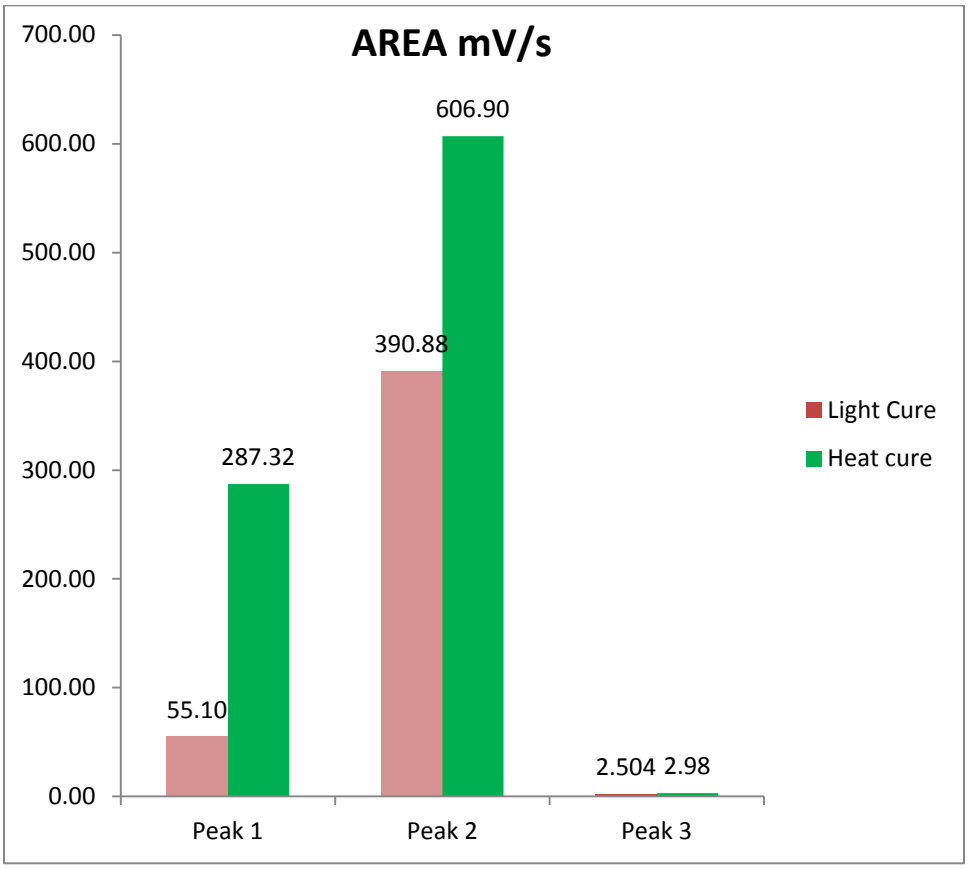
COMPARISON WITH THE STANDARD VALUES IN LIGHT CURE OF RT					
		MEAN	**S.D**	**t-VALUE**	**P-VALUE**
DAY 1	**PEAK 1**	2.464	0.0114	2.746	**0.052***
	PEAK 2	2.822	0.017	-3.125	**0.035***
	PEAK 3	3.528	0.043	-1.293	0.266
DAY 3	**PEAK 1**	2.468	0.008	4.811	**0.009***
	PEAK 2	2.836	0.0296	-0.829	0.454
	PEAK 3	3.532	0.022	-2.059	0.109
DAY 7	**PEAK 1**	2.43	0.0374	-1.195	0.298
	PEAK 2	2.88	0.04	1.845	0.139
	PEAK 3	3.556	0.0477	0.14	0.895
*** SIGNIFICANTLY DIFFERENT WITH STANDARD VALUE**					

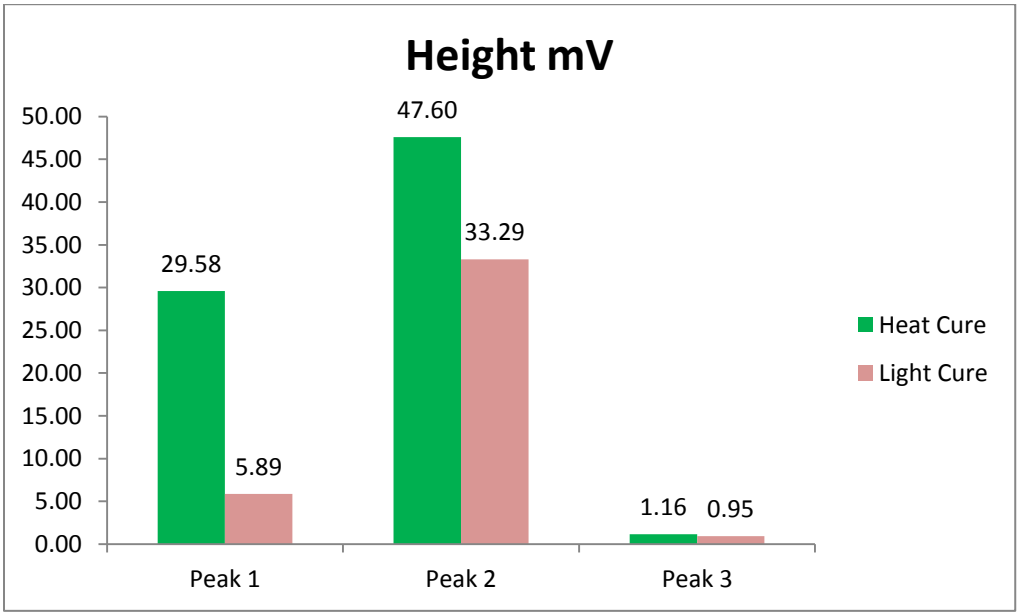

DISTRIBUTION OF LIGHT CURE VALUES
2.464
2.822
3.528
2.468
2.836
3.532
2.43
2.88
3.556
PEAK 1
PEAK 2
PEAK 3
PEAK 1
PEAK 2
PEAK 3
PEAK 1
PEAK 2
PEAK 3
DAY 1
DAY 3
DAY 7


DISTRIBUTION OF HEAT CURE VALUES
2.46
2.846
3.55
2.454
2.864
3.532
2.44
2.856
3.504
PEAK 1
PEAK 2
PEAK 3
PEAK 1
PEAK 2
PEAK 3
PEAK 1
PEAK 2
PEAK 3
DAY 1
DAY 3
DAY 7

ONE HOUR ANALYSIS GRAPHICAL REPRESENTATION OF THE PARAMETERS RETENTION TIME, AREA AND HEIGHT.




AREA mV/s
700.00
600.00
500.00
400.00
300.00
200.00
100.00
0.00
606.90
390.88
287.32
55.10
2.504
2.98
Peak 1
Peak 2
Peak 3
Light Cure
Heat cure


Height mV
50.00
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
47.60
33.29
29.58
5.89
1.16
0.95
Peak 1
Peak 2
Peak 3
Heat Cure
Light Cure

The collected data was analysed with SPSS 16.0 version.

To describe about the data descriptive statistics mean, S.D was used .

To find the significance difference between the standard values and samples of RT, single sample t-test was used .

In the above statistical tool the probability value P=.05 is considered as significant**.**

The results suggest that the Chlorhexidine is releasing in seven different peaks with the major retentive time in the 2.48 and 4.35 mins in the standardization procedure with which the samples are compared.

In comparison to the std chart of chromatography of Chlorhexidine the sample of heat cure's retention time averages to 2.45mins and 2.83min for peak one and peak two.

In comparison to the std the chromatography results of light cure's retention time averages to 2.47 and 2.87 min for peak one and peak two

So now keeping the retention time the areas of peak are compared. The area of the std Chlorhexidine for the retentive time 2.45min the area of chlorhexidine is 1963.39 mV and for3.55 min of retentive time the area is 226.56 mV/s.

In case of heat cure discs the area is 287.3mV/s for first peak ,606.9mV/s for the second peak and 2.9mV/s for the third peak in comparison to std coincides with 2.46 min retention time and 2.85min retention time but a very minimal area of2.98mV in the 3.55 min retention time when compared to std. .

In case of light cure discs the area is only 55.10 in 2.47 retention time, 390.88mV area in 2.84min retention time which is very much minimal to one seen in std .

In same way the height of the first peak is29.58mV and that of the second peak is 47.60mV for the heat cure whereas the std chart that shows height of 116.17mV and 55.86mV.,for heat cure discs.

In light cure discs the height of the first peak is5.89mV and that of the second peak is33.29mV for the heat cure whereas the std chart that shows height of 116.17mV and 55.86mV.,for heat cure.

The results indicate that the retention time and the area are more for heat cure resin than light cure resin discs in comparison to that of standrad of chlorhexidine.

Fig .No. I. LIGHT CURE DISCS BEFORE TREATMENT IN 1000 MAGNIFICATION

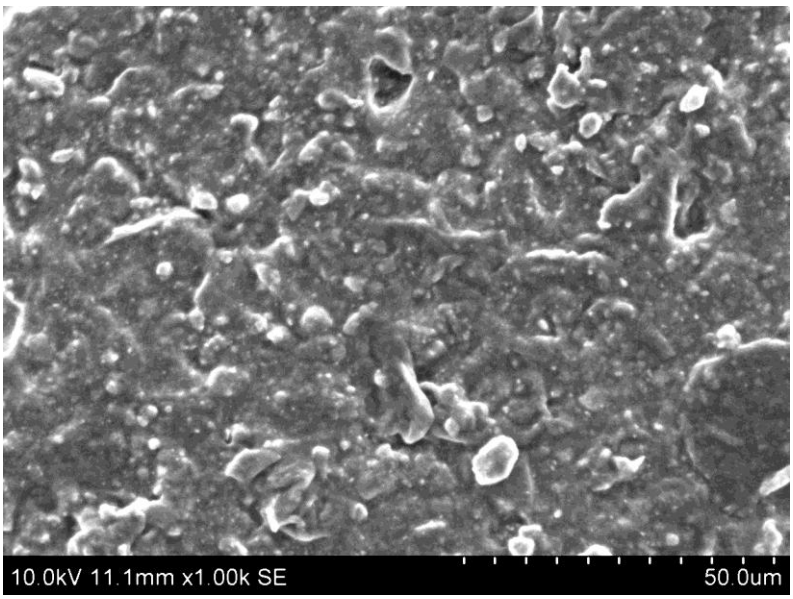


Fig No. II LIGHT CURE DISCS AFTER TREATMENT IN 1000 MAGNIFICATION

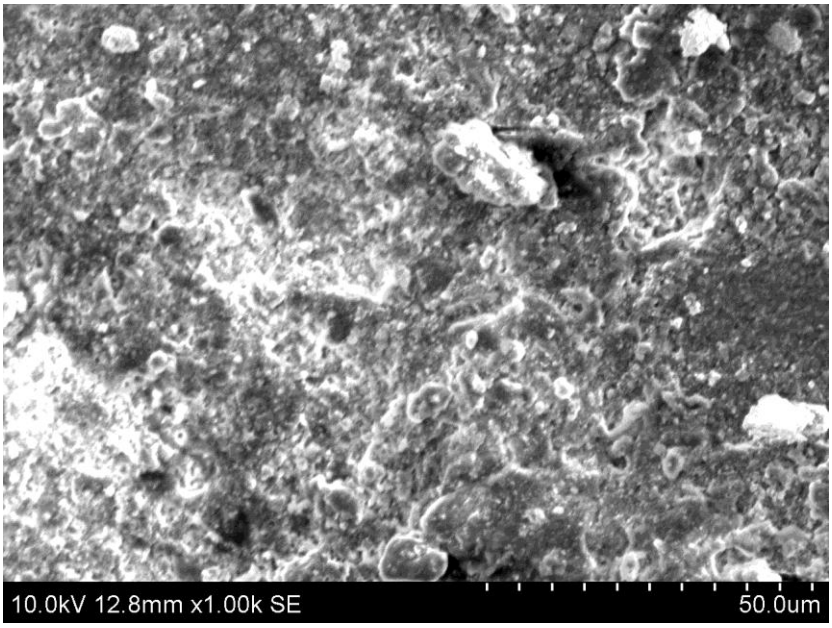


Fig.No.III HEAT CURE DISC BEFORE TREATMENT IN 1000 MAGNIFICATION

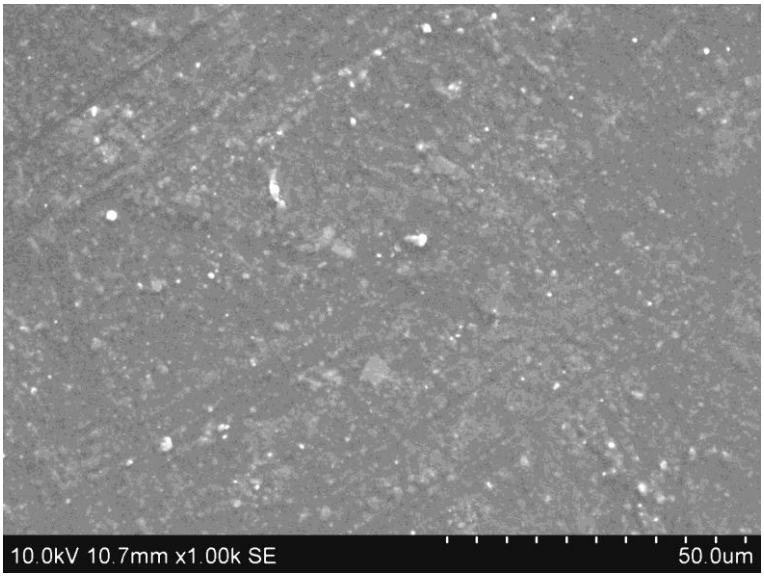
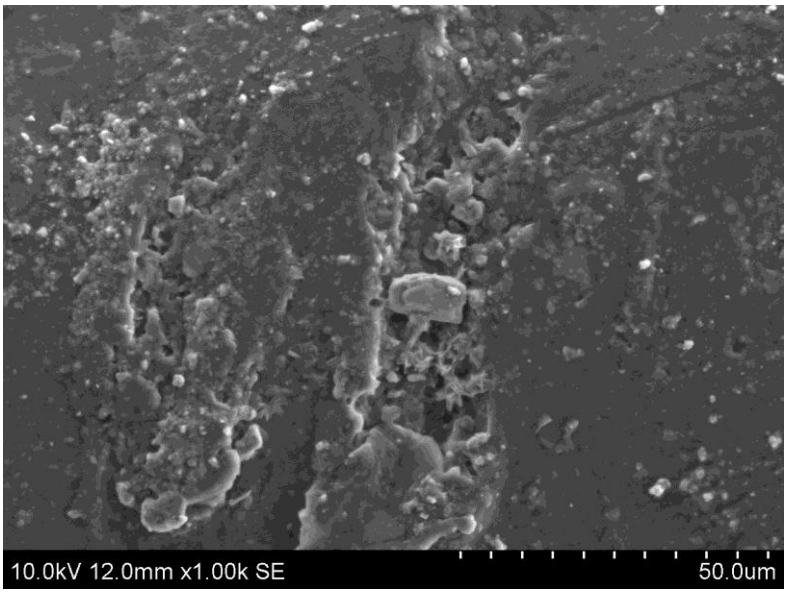


Fig.No.IV HEAT CURE DISCS AFTER TREATMENT IN 1000 MAGNIFICATION



1. Figure one is the SEM photomicrography of light cure discs pre treatment to Chlorhexidine showing surface tophography with irregularities
2. Figure two is the SEM photomicrography of light cure discs pre treatment to Chlorhexidine showing surface tophography with irregularities being deposited with a surface coating.
3. Figure three is the SEM photomicrography of heat cure discs pre treatment to Chlorhexidine showing surface tophography with smooth, clean and regular surface
4. Figure four is the SEM photomicrography of heat cure discs pre treatment to Chlorhexidine showing surface tophography with irregularities on the surface like a coating on the resin disc. Further studies are required to detect what are those surface coatings consisting of and to know their chemical nature.

The occurrence of Candida albicans on the denture base and its impact on the removable denture wearers by causing denture stomatitis has been vastly discussed in literature[24,54,55].

Denture stomatitis and poor oral hygiene causes plaque accumulation and it leads to decrease in the pH of oral cavity to acidic environment which favours Candidal growth.[56.57]Low levels of pH can favour the adhesion and the proliferation of Candida yeast. In fact, a pH equal to three is optimal not only for the adhesion of the yeasts, but also for the enzymatic activity of the proteinases that, together with the lipases, are the most important factors of virulence of Candida because of their cytotoxic and cytolyticeffects.[42]In particular, it can cause persistent , intractable infections in immune compromised patients, diabetic patients, differently abled patients or in patients who have had a local disturbance in their oral flora like those under long term antibiotics.

The treatment of Candida albicans Associated Denture Stomatitis has been evolving from simple emphasis on maintaining good oral hygiene to using new system of slow drug delivering.

Lal et al. investigated the use of Chlorhexidine gluconate in the form of Peridex both as a mouthrinse and a denture soak in the treatment of denture stomatitis. It was found that chlorhexidine completely eliminated Candida albicans on the acrylic resin denture surface and significantly reduced palatal inflammation. However, several weeks after the Peridex treatment was terminated, Candida albicans recolonized the denture surface and palatal inflammation recurred[58.]Likewise in our study too the results suggest that the retention time and area are similar to the standard but it reduces with time and the area also reduces in due course of time.

Chlorhexidine is a cationic chlorophenyl bisbiguanide that binds to negatively charged surfaces. It has a broad spectrum of antimicrobial activity including Candida albicans. Exposure of Candida to Chlorhexidine results in loss of structural integrity, diminished ability to adhere, and fragmentation of the cell wall.

Bonesvoll p,Llokken p,Rollag et al quotedthat after a one min rinse with Chlorhexidine, 30% was retained in the oral cavity for 24 hours.This invitro study has also has proven that the retention time and area as opposed to standard is less and with time the Chlorhexidine release is not there.

Though these acrylic resins are major habitat on which the microorganism are able to flourish well it also has the property of leaching out of chemicals from it. Taking this as the advantage was the various studies conducted to use them as the drug delivery system. It has been clearly established that methacrylate-based polymers absorb up to 30% water depending on the osmolarity of the external solution or the formulation of the particular polymer [38.]

It has been proved that because of the porous nature of acrylic surface the water goes into the surface by slow diffusion process and tries to bring out the chemicals absorbed by the resin[59]. Diffusion based release of residual monomer occurs with the sorption of water into the spaces between the polymer chains[60.]

In this study heat cure and light cure are used. The light cure resin used here is heliomolar flow as it contains both urethane dimethacrylate and triethylene dimethacrylate keeping in mind the study conducted that the unreacted monomers and additives may be released from cured dental resins (Sideridou *etal.*, 2003) and composites (Ferracane, 1994; Örtengren *et al.*, 2001; Michelsen *et al.*, 2003).The quantity of release

can be as high as 2 wt% of the resin component of the composite(Ferracane, 1994) and from 0.2 to 1.4 wt% from cured resins (Sideridou *et al.*, 2003).

So in this study it was tested whether the change in the composition of the resin bought any change in the absorption and release pattern of Chlorhexidine.It was found that the retention time and area were found less than that of both the standard and the heat cure also.

Autopolymerizating acrylic was not used as do not defer much in the chemical nature of heat cure resin in containing polymethylmethacrylate, except that are activated by benzoyl peroxide. Since metal based dentures have shown release of various metallic agents like nickel chromium which was assessed by gas chromatography assessing the peaking of Chlorhexidine would be still more complicated,so auto polymerizing resin,metal based resin were not used in this study.

Therefore it a possibility that constant exposure to Chlorhexidine can cause its absorption in to the resin and further release of the same after some period of time.

There has been various methods evolving in detecting the release of chemicals from the resin. The present studies are done using gas chromatography of which now the high performance liquid chromatography has the added advantage of being non destructive and facilitate sample recovery.

Obtaining of pure samples for standardization was compulsory so the asep rc prep solution was used as it contained only Chlorhexidine of two percentage.In order to standardize the procedure the same asep rc prep was used for immersing the samples.

Therefore this study was undertaken to assess the absorption and release of Chlorhexidine from acrylic resin discs immersed in them overnight simulating the

clinical conditions. The release of the Chlorhexidine was assessed by means of High Performance Liquid Chromatography. To first standardize the procedure the 2% Chlorhexidine is subjected to high performance liquid chromatography analysis. Once this analysis is obtained now the samples are subjected to High Performance Liquid Chromatography analysis. The data obtained from this analysis suggests that with time the peak height and area decreases and the peaking also reduces.

It shows that the release of Chlorhexidine is comparable to the standard in the second peak thus this implying that allowing it exert its antifugal effect in accordance to the study conducted by **Anibal, P.C.** et al Chlorhexidine permits extended activity following exposure in the oral environment and allows a wide spacing of doses.

The release of Chlorhexidine from the resin discs to distilled water indicated that these discs can be used as drug delivery systems. This finding is in agreement with previously reported studies that employed polymers for delivering Chlorhexidine.The results from this study suggests that Chlorhexidine peaks from that of the samples are less in area and height when compared to standard obtained from High performance Liquid Chromatography. Within the two discs used the heat cure discs are comparably having larger area and height for both the peaks than the light cure discs.

The minimum inhibitory concentration of Chlorhexidine required for performing its antifungal property is 7.81µg/ml, these mouthwashs have more than this concentration thus killing the fungus in just 60 seconds of exposure[61,46].Has the added advantage of cleaning the denture as well as treating oral candidiasis effectively.[43.]

Ferguson et al (2002) Chlorhexidine di gluconate exhibited anti fungal effect at <0.63µg\ml. This study concluded that sodium hypo chlorite, hydrogen peroxide, and

Chlorhexidine digluconate diffuse through the root canal and will be effective fungicidal agents, even if they become significantly diluted in the process. Thus supporting this study that the minimal amount released will surely cause antifungal effect.

This study supports that the change in the rate of drug release is attributed to the fact that leaching of Chlorhexidine into water is governed by a concentration-dependent diffusion process, as seen in the study done by **Patel MP, Cruchley AT, Coleman DC, Swai H, Braden M, Williams DM** et al who used new polymeric system to assess the release of Chlorhexidine.

However there are certain limitations in this study, i.e. since this is an in vitro study the results obtained in this study cannot be extrapolated to in vivo environment. Everyday Chlorhexidine rinsing may cause teeth and tongue staining, taste disturbance, and mucosal irritation therefore an alternative mouth washes are gaining popularity that has few, if any side effects, to be more appropriate in patients with oral candidiasis, particularly in immuno compromised persons who received anti fungal agents for long periods of time.

Using scanning electron microscopy has the advantage of viewing the topographical morphological and composition information .The amorphous matrix of polymethylmethacrylate can be visualized from the resin discs before treatment. The irregularities are seen on the surface in the post treatment photographs can be attributed to either deposition of Chlorhexidine or may be only the distilled water in which they have been soaked. Further studies need to be conducted to assess the deposition particles on the resin discs.

The design of the study was formatted in Department of Prosthodontics, Tamil Nadu Govt Dental colleges. Since it was an in vitro study it was carried out in lab of Pharmacovigilance Department,Madavaram Veterinary and Animal Science University.

Acrylic resin discs were immersed in 2% Chlorhexidine solution after which they were washed and immersed in distilled water which was subjected to High Performance Liquid Chromatography analysis and also for Scanning Electron Microscopy analysis.

The results of the this in vitro study suggest that compared to the retention time and the area of a standard chlorhexidine and that of the samples is less but effective enough to perform antifungal effect.

And also the use of these antimicrobials tested in this in vitro project may prove useful in reducing the risk of fungemia in immunosuppressed patients and may lead to other controlled studies to ultimately determine the scope of the clinical significance. Further studies have to be conducted like an invivo study to test the antifungal effectiveness of Chlorhexidine clinically in denture wearing patients especially those prone to develop denture induced stomatitis .Therefore further studies are required to test the effect of these mouth rinses.

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