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EVALUATION THE EFFECT OF INCORPORATED HYDROXYAPATITE PREPARED FROM DRIED EGG SHELL ON SOME PROPERTIES OF RELINED DENTURE BASE

By **Zena Joma Hassan AL-Bahar**

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University of Mosul
College of Dentistry

Evaluation the Effect of Incorporated Hydroxyapatite Prepared from Dried Egg Shell on Some Properties of Relined Denture Base

A Thesis Submitted by
Zena Joma Hassan AL-Bahar

To
The Council of College of Dentistry
Mosul University
In a Partial Fulfillment of the Requirements
For the Degree of Master of Science
In
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ABSTRACT

Objective: Hydroxyapatite (HA) [$Ca_{10}(PO_4)_6(OH)_2$] was used in various biomedical fields such as dental material, bone substitute and hard tissue paste. Hydroxyapatite (HA) reinforced polymer have many potential clinical applications like bone cement, dental implants, coating of joint replacement prosthesis (Tham and Chow, 2010).

So the aims were to determine the effect of incorporation of recently synthetized Hydroxyapatite from egg shell in different concentration on dimensional accuracy, transverse strength, FTIR of RESPAL NF heat cured acrylic denture base, with, and without relining, and biocompatibility test on rabbit.

Materials and Methods: 171 samples of heat cured acrylic resin (HCAR) were prepared, and divided into five groups; the 1st group (Control) heat cured acrylic resin, the 2nd groups heat cured acrylic resin mixed with 2% of Hydroxyapatite (HA), the 3rd groups (HCAR) relined with 2% (HA), the 4th group (HCAR) mixed with 5% of Hydroxyapatite, and the 5th group (HCAR) relined with 5% of Hydroxyapatite.

The samples were tested after 48 hours from preparation for (biocompatibility tests through implantation of samples in subcutaneous layer of the mandible of the local bred rabbit, FTIR test, microscopical examination of Hydroxyapatite, indentation hardness, transverse strength, water sorption and solubility, tensile strength, dimensional accuracy, residual monomer.

Results: showed significant differences in the transverse strength, no residual monomer release, no dimensional changes after adding Hydroxyapatite to (HCAR). FTIR spectra of pure poly methyl methacrylate

(PMMA), and the (PMMA) with Hydroxyapatite extracted from egg shells showed no difference in the peaks before, and after addition of Hydroxyapatite to the polymer. Results of implantation samples showed increased bone formation.

Conclusions: after adding Hydroxyapatite to heat cured acrylic resin showed an increase in strength of denture base with high dimensional accuracy, good biocompatibility with new bone formation in both 2%, and 5% Hydroxyapatite. FTIR spectra of polymer with and without Hydroxyapatite showed homogeneous distribution which will improve the mechanical properties of heat cured acrylic resin.

Introduction

Poly methyle methacrylate has been the most popular material for construction of dentures since the 1930s because of its advantages including good aesthetics, accurate fit, stability in the oral environment, easy laboratory and clinical manipulation and inexpensive equipments (Nejatian *et al.,* 2006).

Bone is a dynamic tissue, in constant resorption and formation, permitting the maintenance of bone tissue, the repair of damaged tissue and the homeostasis of the phosphocalcic metabolism . Through this balanced phenomena, known as the remodeling process (Hernández-Gil *et al.*,2006).

The repair and regeneration of bone is a major issue in the oral maxillofacial filed (OMF) and for the whole human body in general (Salgado *et al.*, 2006).

The aim of tissue engineering (TE) is the regeneration of tissues through the combined use of biomaterials and biologic mediators in order to provide new tools for regenerative medicine (RM) (Bluteau *et al*., 2008).

Hydroxyapatite (HA) [$Ca_{10}(PO_4)_6(OH)_2$] was used in various biomedical fields such as dental material, bone substitute and hard tissue paste. Hydroxyapatite (HA) reinforced polymer have many potential clinical applications like bone cement, dental implants, coating of joint replacement prosthesis (Tham and Chow, 2010).

In order to prepare fine (HA) powders, many chemical processing routes have been employed, including hydrothermal reactions, sol-gel synthesis, pyrolysis of aerosols and micro emulsion, biomimetic process, and chemical precipitation, which is the most used alternative (Gauthier *et al*., 1999).

Nowadays, several improvements in injectable bone substitutes are being developed as minimally invasive cell carriers for tissue regeneration both for bone and cartilage alternative (Gauthier *et al*., 1999; Tham and Chow, 2010).

Desired characteristics of synthesized Hydroxyapatite (HA) are fine and uniform particle size, in the nanometer range, phase homogeneity and minimized degree of particle agglomeration (Best *et al*., 1989 ; Termenoff and Mikos, 2000).

Bone exhibits natural Hydroxyapatite (HA) crystals with needle-like or rod-like shapes, well arranged within the polymeric matrix of collagen type I (Liou and Chen, 2003).

These natural nano particles formed in physiological environment have a more dynamic response when compared to synthetic material with larger particle size (Nejatian *et al*., 2006).

Aims of Study

The aims of this study were to evaluate the followings :

1. Biocompatibility of Hydroxyapatite (2%,5%) extracted from egg shell on rabbits.
2. The effect of incorporation of Hydroxyapatite (2%,5%) extracted from egg shell on some mechanical properties (Identation hardness test, Transverse strength, Tensile strength), physical properties (Water sorption and solubility, Dimensional accuracy and Microscopical examination of prepared polymer) and chemical properties (residual monomer and FTIR) properties of heat cured acrylic resin denture base mixed or relined denture base.

CHAPTER TWO

REVIEW OF LITERATURE

2.1. Acrylic Resin Denture Base Material

History

In 1937, poly methyl methacrylate (PMMA) was introduced and used widely as a denture base material. PMMA provided enhanced physical and esthetic properties; in addition, it was readily available, inexpensive and relatively eases of use, and reliance on simple processing equipment. Materials based on (PMMA) are the most common ‘plastics’ used in the dental laboratory, where their uses include the production of soft linings for dentures, together with close-fitting impression trays, orthodontic devices and the repair of dentures. The acrylic plastic represents an estimated 95% of plastics used in prosthetics (Phoenix, 1996 ; Ray, 2001; Criag *et al.,* 2004; Meng and Latta, 2005).

2.2. Relining:

Relining is the process of adding some materials to the tissue side of denture to fill the space between the tissue and the denture base (Robert *et al*., 1985; Levin and Richared, 2002).

2.2.1. Indications of Relining:

a. Immediate dentures at three to six months after their original construction.

b. When the residual alveolar ridges have resorbed and the adaptation of the denture bases to the ridge is poor (Orthman and Ortman, 1975; Knechtel and Loney, 2007).

c. When the patient cannot afford the cost of having new dentures constructed.

d. When the construction of new dentures with the companying series of appointments can cause physical or mental stress, such as for geriatric or chronically ill patient (Robert *et al*., 1985; Knechtel and Loney, 2007).

2.2.2. Contra indications:

1. When an excessive amount of resorption has taken place.
2. When abused soft tissues are present .The relining is not indicated until the tissues recover and return as closely as possible to normal form.
3. When the patient complains of temporomandibular joint problems. Until accurate diagnosis and treatment of the problem has been accomplished, relining is contra indicated.
4. If the dentures create a major speech problem.
5. When severe osseous undercuts exit, until surgical removal and healing occurs (Robert *et al*., 1985).

2.3. Procedure of Relining:

1. Making an impression of denture as a tray, reflasking the denture, removing the impression material, and packing and curing the new liner.
2. Making a chair side reline in which the reline material is used to make the impression (Robert *et al*., 1989).

2.4. Materials used for relining:

Two different types of reline materials may be used. One is designed as a permanent reline, and the other is used only as temporary reline. The former may be either a heat accelerated, chemically accelerated, or light-activated acrylic (Robert *et al*., 1989; Tandon *et al*., 2010).

2.4.1 Permanent Reline Materials:

2.4.1.A. Heat Cured Acrylic:

Poly methylmethacrylate is the most widely used denture base material. Properly handled during technical procedures it meets the exacting requirements of the oral environment satisfactorily and is more economical and versatile than any other current base materials. Its use in relining assures the continuation of its advantages such as ease and accuracy of molding, density, color stability, strength, esthetics and tissue compatibility (Orthman and Ortman 1975; Wyatt *et al*., 1986; Matsumura *et al*., 2001; Yatabe *et al*., 2001;Machado *et al*., 2002).

2.4.1.B. Light Activated Reline:

The light activated material is used directly in the denture to record the tissue surface. A bonding agent is used before placing the material in the denture. Polymerization is achieved in alight chamber. The light activated acrylic allows the entire reline procedure to be completed in 30 to 45 minutes (John *et al*., 1978; Zissis *et al*., 2001).

2.4.2. Temporary Relining Material:

Temporary relining plastics are used directly in the mouth, and a chemically accelerated curing system is used. These materials are considered temporary because they are porous and stain easily or foul. In addition ,most of these products are not color stable. These temporary reline materials polymerize at mouth temperature, with peak polymerization temperature of 59°C to 79°C and peak temperature times of 6 to 11 minutes, with lower temperature generally corresponding to the longer times. Peak temperatures of 79°C are certainly uncomfortable. To avoid burning of the tissues, the denture is usually taken from the mouth after a few minutes, chilled in cool water, and returned to the mouth .In addition to the heat, direct contact of monomer the oral tissues may elicit a burning sensation (Robert *et al*., 1989; Zissis *et al*., 2001).

2.4.2.A. Chair side Relining:

Relining usually requires that the patient should be without his denture for about twenty-four hours while the laboratory procedures are being completed. Unless the patient has spare denture, this often creates a problem. One way to help the patient avoid this complication and speed the procedure is chair side relining. Chair side relining materials presently available, however, have disadvantages:

1. Their porosity allows them to become discolored and pick up odors.
2. Either the chemistry of the material or the heat of polymerization may burn the patients oral tissues.
3. If the denture is not correctly positioned while relining error are almost impossible to correct (Charles *et al*., 1975; Knechtel and Loney, 2007).

2.5. The problems involved in relining a denture (Charles *et al.*, 1975):

a. A good chemical bond is desired between the reline plastic and the denture plastic.
b. Satisfactory strength of relined denture is necessary.
c. No wrapage or dimensional change should result in the denture because of relining procedures.
d. For patient convenience the relining should take as short a time as possible.

2.6. Etiology of Residual Bone Resorption:

Etiology of residual bone resorption (RRR) is a multi-factorial, biomechanical disease that results from a combination of anatomic, metabolic and mechanical determinants. Since all of these factors vary from one patient to the next, these different cofactors may combine in infinite variety of ways, thus explaining the variations in etiology of residual bone resorption between patients (Atwood, 1971; Prithviraj *et al*, 2008).

2.6.1. Anatomic Factors:

Etiology of residual bone resorption, anatomic factors i.e. amount of bone and quality of bone. Amount of bone; It is not a good prognostic factor for the rate of (RRR), because it has been seen that some large ridges resorb rapidly and some knife edge ridges may remain with little changes for long periods of time. Although the broad ridge may have a greater potential for bone loss, on theoretic grounds, the rate of vertical bone loss may actually be slower than that of a small ridge because there is more bone to be resorbed per unit of time and because the rate of resorption also depends on the density of bone (Winkler, 1979; Prithviraj *et al*, 2008).

2.6.2. Metabolic Factors:

Etiology of residual bone resorption; bone resorption factors Bone formation factors, general body metabolism, is the net sum of all the building up (anabolism) and the tearing down (catabolism) going on in the body. In equilibrium, the two antagonistic actions of (osteoblasts and osteoclasts) are in balance. In growth, although resorption is constantly taking place in the remodeling of bones as they grow increased osteoblastic activity more than makes up for the bone destruction. Whereas in osteoporosis, osteoblasts are hypoactive, and, in the resorption related to hyperparathyroidism, increased osteoblastic activity is unable to keep up with the increased osteoclastic activity (Wyatt, 1998).

The normal equilibrium may be upset and pathologic bone loss may occur if either bone resorption is increased or bone formation is decreased, or if both occur. Since bone metabolism depends on cell metabolism, anything that influences cell metabolism of osteoblasts and osteoclasts is important (Wyatt, 1998; Zatarić *et al*., 2002).

In general terms, anabolism exceeds catabolism during growth and convalescence, levels off during most of adult life and is exceeded by catabolism during disease and old age. Bone has its own specific metabolism and undergoes equivalent changes. At no time during life is bone static, but rather it is constantly rebuilding, resorbing and remodeling subject to functional and metabolic stresses (Remodelling, 1998; Wyatt, 1998; Manoj *et al.,* 2011).

2.6.3. Functional Factors:

Functional factors include the frequency, intensity, duration and direction of forces applied to bone which are translated into cellular activity, resulting in either bone formation or bone resorbtion, depending upon on the patient's individual resistance to these forces. When force within certain physiologic limits is applied to living bone that force, whether compressive, tensile of shearing, brings about by some unknown mechanism the remodeling of bone through a combination of bone resorption and bone formation (Brudevold, 1969).

Masticatory and non-matiscatory force is ordinarily transmitted to the dento-alveolar bone through the periodontal ligament. Once the teeth are removed, the residual ridge is subjected to entirely different types of forces. Some postulate that etiology of residual bone resorption is an inevitable “disuse atrophy”. Others postulate that etiology of residual bone resorption is an “abuse” bone resorption due to excessive forces transmitted through dentures. Perhaps there is truth in both the hypotheses (Atwood and Coy, 1971, Schropp *et al* 2003).

2.6.4. Prosthetic Factors:

Ridge resorption may or may not occur in patients for whom dentures are not made. If resorption does occur, it is attributed to disuse atrophy an atrophying mucosa seeking a reduced area, thereby causing pressure resorption of the ridge. If resorption does not occur, this is attributed either to function by a patient who is able to “gum” food because of a small inter-ridge space or unknown factors. The prosthetic factors are extremely difficult to evaluate because of tremendous number of variables, including anatomic, metabolic and functional factors. The traditional design of dentures includes many features whose goal is to reduce the amount of force to the ridge and to thereby reduce etiology of residual bone resorption (Atwood *and* Coy, 1971).

These prosthetic factors include broad-area coverage (to reduce the force per unit area); decreased number of dental units, decreased buccolingual width of teeth, and improved tooth form (to decrease the amount of force required to penetrate a bolus of food) (Atwood, 1963 ; Enlow *et al*., 1976).

Avoidance of inclined planes (to minimize dislodgement of dentures and shear forces), centralization of occlusal contacts (to increase stability of dentures and to maximize compressive forces), provision of adequate tongue room (to increase stability of denture in speech and mastication); adequate interocclusal distance during rest jaw relation (to decrease the frequency and duration of tooth contacts) etc. Various clinical studies have attempted to correlate one or more of these factors with the rate of Etiology of residual bone resorption (Atwood, 1963 ; Enlow *etal*., 1976, Manoj *et al*., 2011).

2.7. Some Method to Improve Residual Bone:

The ultimate goal of a ridge augmentation procedure is to form a bearing surface for the denture that will provide stability, retention, and support. Residual ridge reconstruction with an implanted material must be based on an understanding of the variations of ridge atrophy. A problem-oriented classification describes the different forms of residual ridges. This classification of similar types of resorbed residual ridges can be used to establish a rational plan of treatment. The proper placement of hydroxyapatite is critical to obtain the desired reconstructed residual ridge. In the mandible, a lingualized placement of the alloplast is suggested. Careful use of an anatomic matrix to support and contain the hydroxyapatite particles eliminates the need for a splint. The surgical stage includes vestibuloplasty and skin grafting to provide the patient with an increased denture-bearing surface. The ridge created in the form of a comma posteriorly enhances retention and stability. The broad residual ridge with an improved interarch relation also enhances the support and provides a better foundation for balanced occlusion(Zeltser *et al*., 1989).

Replacement of anterior teeth with fixed or removable prostheses is often a compromise, because the resorbed residual ridge in the area of missing teeth cannot be ideally restored functionally and esthetically at the same time. To address this problem, a blocks of hydroxyapatite were placed subperiosteally to improve residual ridge resorption defects subsequent to loss of anterior teeth. The implants were evaluated clinically and radiographically after implantation. Results indicate that the suggested approach improves the esthetic results and the prognosis of fixed prostheses(Wijs *et al*., 1993).

2.8. Denture Base as Drug Carrier:

Several researches investigated the feasibility of using drug delivery system by incorporation of antifungal or antimicrobial agents, with denture acrylic resin (Budtz-Jorgensen and Carlino, 1994) or with soft liners(Nikawa *et al*., 1997). The idea suggested the use of polymerized acrylic as carriers for drugs orally (Douglas, 1977). Similarly, soft liners placed in dentures have been used as carriers for antifungal drugs in treating denture stomatitis (Addy and Handley, 1981).

These formulations have been claimed of having less side effects compared with the conventional forms, because of the continual presence of the drug at the site of action, less amount of drug is needed to achieve the therapeutic effect (Garcia *et al*.,1978). They were also believed to be of high benefit for physically or mentally compromised patients (Mirth *et al*., 1989). An example of these formulations, in which sustained topical administration of chlorhexidine via a self-cured polymeric system based on polyethyl methacrylate and tetrahydrofurfuryl methacrylate (PEM/THFM) had been advocated (Rigges *et al*., 2000)**.**

The choice of the polymeric system was based on its abundance, ease of handling and cost effectiveness. The room-temperature polymerizing resin version was employed because it has been proven that this mode of polymerization does not adversely affect the efficacy of the antifungal drugs (Patel *et al*., 2001). The employed polymer was intended for use as an indirect relining resin in order to avoid the possible thermal trauma to the denture bearing mucosa that may be caused by the polymerization exotherm (Patel *et al*., 2001).

2.9. Some Mechanical Properties of Acrylic Resin

2.9.1. Hardness:

Hardness is the resistance of a material to indentation (Craig *et al*., 2004).

The low hardness number of acrylic resin base material indicates that these materials may be scratched easily and abraded (Craig and Ward, 1997).

Hardness has been found to be sensitive to the residual monomer content in the polymerized resins, and hardness is an effective method to evaluate the polymerization depth of resin materials. In addition, hardness has been used to predict the wear resistance of dental materials (Craig and Powers, 2002).

Vuorinen *et al*., (2008) concluded that the addition of rigid rod polymer (RRP) particulate fillers increased surface micro-hardness (Vickers hardness) of the acrylic resin.

The addition of Thyme and Nigella sativa oils increased the hardness of the denture base materials. The addition of Thyme oil and Nigella oil with concentration 1%, 1.5%, and 2% showed an increase in the hardness of the denture base, and no significant difference with different concentration of oil, this is due to un reacted monomer with coated polymer with oil (Hatim and Taqa, 2009).

All forms of metal and fiber reinforcement acted to reduce the hardness of acrylic denture base resin (Kasab Bashi and Al-Nema, 2009).

2.9.2. Transverse Strength:

The transverse strength of a material is a measure of stiffness and resistance to fracture.

$$\mathbf{Stress = 3Pl / 2bd^2}$$

Where the (L) is the distance between supports, (b) is the width of specimen and (d) is the depth (thickness) of specimen (Ray, 2001).

The transverse strength of heat-polymerized denture base resin was considerably enhanced by including either metal wires or glass fibers. Moreover, the flexural strength of specimens reinforced with continuous unidirectional glass fibers was significantly higher than that of metal wire or woven fiber reinforcements (Vojdani and Khaledi, 2006).

Kanie *et al*., (2006) compared unreinforced and glass fiber reinforced acrylic resin polymers prepared under both conventional heat curing and microwave curing techniques. Strengthening with the fibers decreased the flexural strength of the resins but increased flexural resistance. Thus when high impact acrylic resins are needed, fiber reinforced resins may be the material of choice.

The transverse strength of Metrocryl high-impact (HI) denture base resin can be increased significantly by a factor of 29% and 76% when reinforced with zirconia in a concentration of 5% and 15% respectively (Ayad *et al*., 2008).

Ozlem *et al*. (2010) indicated that denture base materials and the curing method used influence the transverse strength of repair denture base resins. These variables should be considered by the clinician when communicating with the dental laboratory to ensure maximum strength of the completed prosthesis. Study curing of the denture in temperature controlled water bath along with the use of high-impact heat-activated denture base reins as a repair material demonstrated the highest transverse strength values.

Microwave-cured acrylic resin showed the highest mean transverse strength value, whereas visible light-cured acrylic resin showed the lowest. Heat-cured resins had slightly lower transverse strength values than microwave-cured resin (Unalan *et al*., 2010).

2.9.3 Water Sorption and Solubility

Sorption of the material represents the amount of water absorption on the surface and into the body of the material, the sorption of poly methyl methacrylate (PMMA) is facilitated by its polarity and the mechanism primary responsible for ingress of water is diffusion (Anusavice, 1996).

The rate at which the materials absorbed water or lost soluble components varied considerably with the type of material, the amount of the plasticizer or filler content and the solution in which they were immersed (Kazanji and Walkinson, 1988).

Heat-cured PMMA denture base resin showed the highest wettability, therefore, it can be suggested that heat-cured PMMA resin should provide superior denture retention and patient comfort to self-cured PMMA and silicone denture relining material (Na-young *et al*., 1988).

Sorption can be related to the polarity of acrylic resin(due to unsaturated bond) (Philips, 1991). While, solubility can be related to the leach of soluble material, that present in an acrylic resin, (initiator, plasticizer, residual monomer) (Takashi *et al*., 1995).

The results of sorption and solubility tests showed that there was no statistically difference between the two curing methods (water bath and microwave energy) (Anusavice, 1996).

The rise in immersion temperature increased the diffusion of water but did not have a significant effect on the maximum water uptake (Unemori *et al*., 2001).

Solubility represents the mass of the soluble materials from polymer (Craig and powers, 2002).

Although the dimensional change that occurs during polymerization shrinkage may be partially compensated by water absorption, by the resilience of the gingival mucosa and by the saliva film between denture base and the soft support tissue, it is an essential and critical factor in the retention and stability of the denture (Consani *et al*., 2003).

Craig *et al*., (2004) ; Meloto *et al*., (2006) found that the microwave group samples showed a lower sorption than the water bath group samples. Water absorbed by acrylic resin stays in gaps among the interpolymeric chains that form acrylic resin structure. The magnitude of these interpolymeric gaps determines the amount of water to be absorbed. Better polymerization of acrylic resin increases the cross linking and reduces water sorption values (Meleto *et al*., 2006).

2.9.4. Tensile Strength:

Tensile strength is defined as the resistance of the material to a tensile or stretching force. While tensile stress mean the internal induced force that resists the elongation of a material in a direction parallel to the direction of the stresses (Academy of Prosthodontic, 2005 ; Craig and Ward, 1997).

Dogan *et al*., (1995) evaluated the tensile properties of denture base material related to the effect of level of residual monomer, and concluded that the percentage of higher levels of residual monomer affected on the tensile properties of denture base material .

Polyzois, (1995) showed that the tensile strength reveled significant difference among the tested groups and showed that the specimens of heat-cure acrylic resin with metal wire has higher mean value of tensile strength than specimens of heat-cure acrylic resin without metal wire This may be due to adding of metal wire gave support to acrylic materials and gave higher tendency and ability with stand the higher strength.

Evelin *et al*., (1999) suggested that the addition of zinc-neutralized ionomer can produce significant increases in modulus, at only a modest cost in elongation to break .

Polymerizing material under pressure can improve its tensile strength and stiffness. However, the pressure needed for the procedure is material dependent (Brosh *et al*., 2002).

Urban *et al.*,(2007) concluded that the post-polymerization treatments by water-bath at 55°C and microwave can improve the mechanical properties (tensile strength) and biocompatibility for acrylic denture base resin due to promoting reduction residual monomer content .

Heat cured denture base material exhibited higher tensile strength as compared to self-cure denture base material (Arora *et al*., 2011).

Marra *et al*. (2009) observed that thermocycling resulted in a denture base acrylic resin and Biotin denture teeth Conversely.

2.9.5. Dimensional Accuracy

Dimensional change is usually expressed as a percentage of an original length (linear dimensional change) or volume (volumetric dimensional change). Volumetric dimensional change is assumed to be three time than the linear dimensional change for a specific material (Criag and Ward,1997).

The changes in dimensions will vary according to the thickness of resin undergoing polymerization and will depend on the location within flask (Wolfaardt and Jones, 1986).

Dimensional changes during water immersion are closely related to water uptake (Cheng *et al*., 1993).

Xediek *et al*., (2004) showed that there was a statistically significant difference between the packing methods with the best results for the Soli-Rock class III dental stone system packing method.

The resin record bases are processed by microwave curing method had no significant differences in dimensional accuracy when compared to the conventionally processed bases (Yadav, 2011).

All acrylic materials showed linear changes immediately after curing and after finishing and polishing (Arora *et al* ., 2011).

Al-Kafaji, (2011) found that bench cooling of the flask for 1h and 4h for the all intervals times of measurements (immediate, 2 days, 7 days and 30 days) produced the best dimensional stability of the microwave curing acrylic resin, this may be due to the residual internal stress which generated from the polymerization shrinkage and thermal shrinkage during the processing of the acrylic.

2.9.6. FTIR Test

During curing of acrylic resin, polymerization is initiated by free radicals from the benzoyl peroxide. As polymerization proceeds, the reaction never reach 100% conversion i.e. conversion of monomer into polymer is not completed. The degree of conversion is the most important criterion that account for unreacted residual monomer levels. (Nomoto *et al*., 2006).

The final degree of conversion of a resin depends on the chemical structure of the dimethacrylate monomer and the polymerization conditions i.e., atmosphere, temperature, light intensity and photo initiator concentration (Urban *et al*., 2007).

The degree of conversion is expressed as percentage of un reacted C=C bonds (Parikh, 1974).

Materials can be easily analyzed by Fourier transform infrared spectroscopy (FTIR) without the need to reduce the particle size or dilute with Potassium bromide powder (KBr), allowing the analysis of biomaterial in a physiological condition. (Rheman and Bonfield, 1997).

The degree of conversion of dimethacrylates may be improved if the distance between the methacrylate groups is long, and the molecular weight is high, respectively. High conversion is not a goal in itself, however. if the monomer is very flexible, and not sufficiently bulky, the degree of conversion will be high, but the mechanical properties will be poor (Blagojevic and Murphy. 1999).

Water bath and microwave post polymerization (The material was polymerised in a microwave oven and the kinetics of release of residual monomer in water was evaluated by spectrophotometric method up to 24 h) treatments decrease the residual monomer of auto polymerized acrylic resin. (Azzarri *et al*., 2003).

Fourier transform infrared spectroscopy (FTIR) is a widely used analytical technique that is routinely applied to the characterization of biomaterials. However, preparing samples of biomaterials for infrared Spectroscopy is often a tedious process. The main sampling problem in FTIR characterization of biomaterials is that nearly all solid materials are too opaque in their normal forms for direct transmission analysis in the mid-infrared region. This problem can be solved by reducing the optical density of samples to a suitable level by employing various sampling techniques (Cekic –Negas *et al*., 2008). These procedures, however, can alter the nature of the sample and are time consuming. Limited amount of information is available within the near infra-red spectral-region, whereas the mid infra-red region provides most spectral bands for required characterization. So photo acoustic sampling (PAS) provide solution to these problems and the materials can be easily analyzed by PAS-FTIR sampling without the need to reduce the particle size or dilute with KBr, allowing the analysis of biomaterial in a physiological condition.

2.10. Hydroxyapatite (HA):

Hydroxyapatite, also called Hydroxyapatite (HA), is a naturally occurring mineral form of calcium apatite with the formula $Ca_5(PO_4)_3(OH)$, but is usually written $Ca_{10}(PO_4)_6(OH)_2$ to denote that the crystal unit cell comprises two entities. Hydroxyapatite is the hydroxyl end member of the complex apatite group. The OH^- ion can be replaced by fluoride, chloride or carbonate, producing fluorapatite or chlorapatite. It crystallizes in the hexagonal crystal system. Pure Hydroxyapatite powder is white. Naturally occurring apatites can, however, also have brown, yellow, or green colorations, comparable to the discolorations of dental fluorosis. Up to 50%

of bone is made up of a modified form of the inorganic mineral Hydroxyl appetite known as bone mineral (Junqueira and Carniero. 2003).

Hydroxyapatite can be found in teeth and bones within the human body. Thus, it is commonly used as a filler to replace amputated bone or as a coating to promote bone in growth into prosthetic implants. Although many other phases exist with similar or even identical chemical makeup, the body responds much differently to them. Coral skeletons can be transformed into Hydroxyapatite by high temperatures; their porous structure allows relatively rapid in growth at the expense of initial mechanical strength. The high temperature also burns away any organic molecules such as proteins, preventing an immune response and rejection(Kundu *et al*., 2010).

On account of its chemical similarity with the biological calcified tissue it is remarkably biocompatible (Ohtsuki *et al*., 1992).

(HA) is also a potential implant material due to its excellent osteoconductive properties (Jarcho, 1981).

(HA) has been shown to stimulate osteoconduction and is a material that can be integrated into bone without provoking an immune reaction. The biological response to (HA) implants is influenced by its properties. The application of (HA) as useful biocompatible materials largely depends on the purity and morphology of the powder. (HA) can be prepared by different routes like chemical precipitation, sol-gel route, combustion synthesis, synthetic body fluid (SBF) etc. (Arita *et al*., 1995).

The purity in the final (HA) powder and stoichemetry (molar ratio of Ca/P = 1.67) can be well controlled in chemical precipitation route.The different chemical processes use precursors like $Ca(NO_3)_2$, $Ca(OH)_2$ etc. as the source of Calcium[Ca] and $(NH_4)_2$ HPO_4 , $H3PO_4$ etc. as the source of Phosphorus [P] during synthesis of (HA). The extremely pure (HA) powder is very costly and needs high quality precursors. The most of the sources of Ca^{2+} contains different types and level of impurities mainly silica (Adak *et al*., 2010).

The composition of human bone is an inorganic/organic hybrid consisting of 70% (wt) apatitic calcium phosphates and 30% (wt) organic (largely collagen) The apatitic calcium phosphate of bone mineral consists of carbonate, small amount of sodium, magnesium and other trace elements (Cowin *et al*., 1987).

The submicroscopic crystals of calcium phosphates in bone resemble the crystal structure of synthetic (HA). Research of organic polymer substrates on which calcium phosphate (Hydroxy$_{\text{apatite}}$) is induced to form has become active due to the insights such investigations can give into the biomineralization processes in the body. The application of such knowledge may also allow the design of materials which combine strength, elasticity and other remarkable properties. A number of experiments focused on how the concepts of morphogenesis, self-organization and replication are available and could be useful for devising new synthetic strategies (Sinha *et al*., 2001).

The most common is the phosphate and carbonate salts of calcium that are used in conjunction with organic polymers such as collagen and chitin to give structural support to bones and shells. In these structures , the inorganic crystals are laid down in orderly arrays in association with a matrix of organic macromolecules. The influence of organic macromolecules is important in the regulation of growth of the mineral and in the resulting crystal morphology specificity . The nucleation and growth of inorganic compounds on/in an organized multi-component system can also induce changes in the local structure and phase behavior, such that new morphological patterns develop from existing architectures.(Otsuki *et al*., 1993).

It can be concluded that (HA) can be synthesized by various method like precipitation, sol- gel approach, hydrothermal technique, multiple emulsion technique, biomimetric deposition technique, electrode position technique. Scientists and researchers are engaged in solving various challenges related with synthesis (HA) with optimum properties for the use in various biomedical applications (Nayak, 2010).

Scanning electrical micrographs (SEM) showed that the ball milling process resulted in micrometer sized coagulated coarse grains with smooth surface, whereas attrition milled samples are characterized by nanometer size grains. This characterized by nanometer size grains. This characteristic morphology being preserved even after firing at high temprature (900°C). Contrary to ball milling attrition resulted in nanosized and homogeneous (HA) even after milling(Gergely *et al*., 2009).

CHAPTER THREE

Materials and Methods

3.1. Materials:

Materials and Drugs used in this study are listed in Table(3.1.).

Table(3.1.) Materials Used in the preparing samples of this Study

No.	Material	Manufacture	Batch number	Exp.date
1.	Dental stone.	Elite stone (Zehrmack)/Italy	6873	2014
2.	Heat cured acrylic resin.	RESPAL NF(RESINA PALATIA CALDO/Italy)	126837	2016
3.	Hydroxyapatite	Prepared from egg shell		-
4.	Separating medium.	Isodent	1753803	2016
5.	Formaldehyde	3A MEDES company, Korea		-
6.	Hard elastic foils 3mm 2.5mm 1.5mm	Rotex medica Gmbh	1824	2013
7.	Ketamine hydrochloride general anasthesia	Germany		2013
8.	Xylazine(sedative analgesic)	Holland	Horsterweg 26A	2016
9.	Absolute Alcohol	England		2016
10.	Pure vasline	Turky		-

3.2. Instruments

1. Beakers of different sizes.
2. Brush (No.0).

3. Dental metal flask(Ash, England).
4. Glass cylinder measure.
5. Glass jar .
6. Rubber bowel.
7. Stainless steel spatula.
8. Surgical kit, blade No. 15,3.0 silk suture.
9. Tweezers.
10. Wax knife.

3.3. Equipments

1. Autoclave, Hirayamafg corp/Japan.
2. Digital scanner, Canon/China.
3. Domestic micro wave oven, LG / Korea.
4. Digital camera, 8 mega pixel, Canone, (China).
5. Digital Tensile strength tester (Gunt. Universal anterior tester).
6. Digital vernier. (China) accuracy 0.01mm.
7. Electric pH meter, Hanna instrument /Romania.
8. Electrical sensitive digital balance, Satrorius/Germany.
9. Heater, Termikel /Turkey.
10. Hydrolyic Press, Hydrofix BEGO/ Germany.
11. Incubator, Fisher Scientific / Russia.
12. Motic digital microscope / China
13. Reflecting light microscope (Lomo Micmed 2 ,Russia).
14. Rockwell hardness tester , Brooks / Germany.
15. Spectrophotometer (Cecil 2000).
16. Thermostatistically controlled water bath, Derotor, multicure/England.

17. Three point transverse testing machine (Inc. Model CN 472 EVANSTON I11-USA).

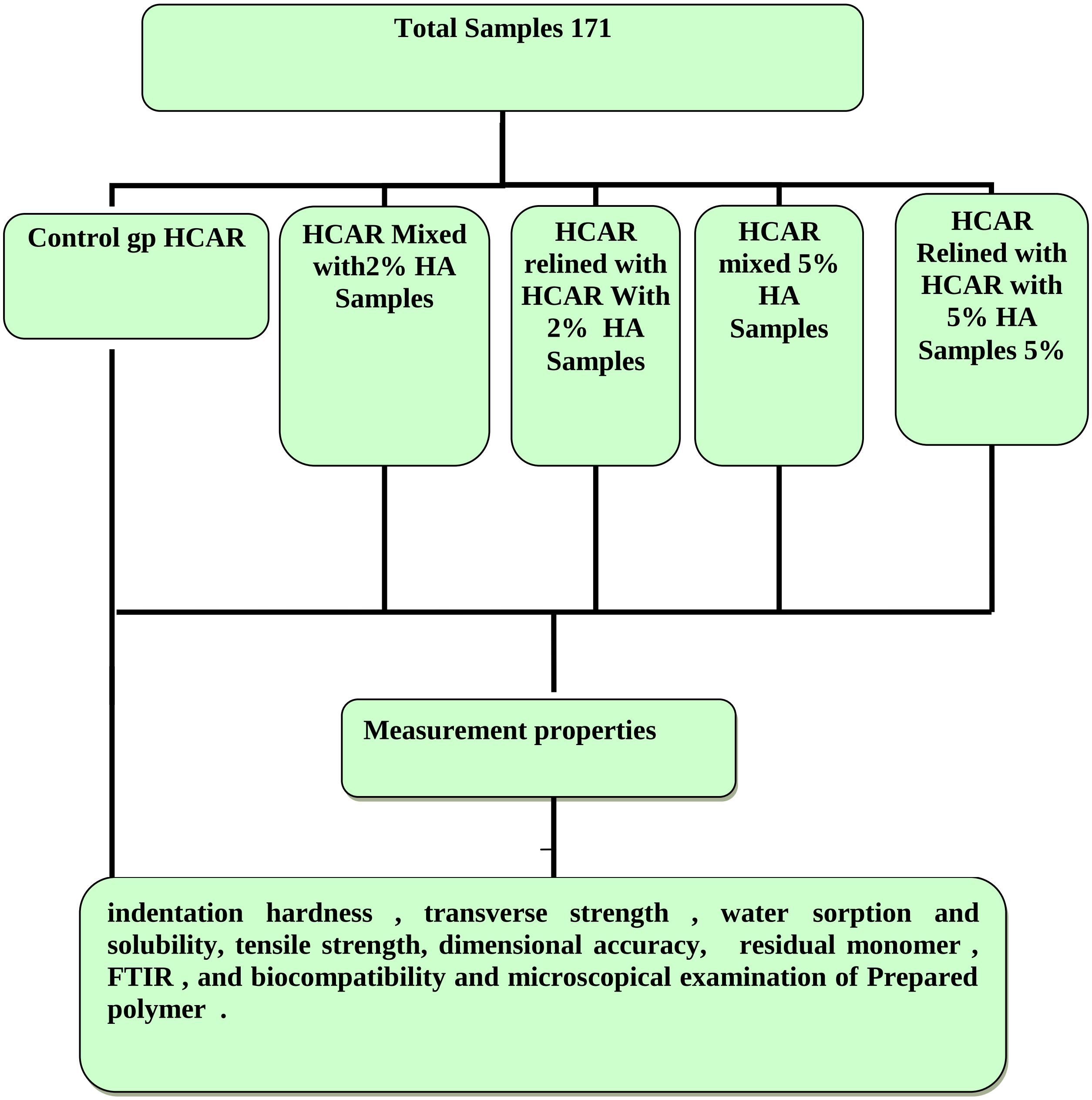
3.4. Main Study

3.4.1. Experimental Design of the Study

Total samples for this study is one hundred and seventy one. The samples were divided into five groups:

1. First group of samples made from heat cure acrylic resin (control group 41samples).
2. Second group of samples made from heat cured acrylic resin with additive 2% hydroxyapatite (39 samples).
3. Third group of samples made from heat cured acrylic resin with additive 5% hydroxyapatite (39 samples).
4. Forth group of samples made from heat cured acrylic resin relined with with additive 2%hydroxyapatite (25samples).
5. Fifth group of samples made from heat cured acrylic resin relined with additive 5%hydroxyapatite (25 samples).

The samples were incubated in distilled water at 37±1°C for two days for conditioning before testing(ADA specification No.12 1975). Immersion samples were tested to determine biocompatibility, FTIR, Microscopical Examination of (HA), indentation hardness, transverse strength, water sorption and solubility, tensile strength, dimensional accuracy, residual monomer, and as shown in Figure (3.1).



HCAR : Heat Cured acrylic resin. HA : Hydroxyapatite .

Figure 3.1:Experimental design of main study for each test

3.4.2 Synthesis of Hydroxyapatite (additive)

Synthesis of Hydroxyapatite from chicken egg shell; all chicken eggs used in this research were selected from the local markets randomly. Just white egg to avoid unwanted color output.

Egg casings were removed for internal crust then cover was lifted lining of the peel and then wash away the chaff very well to be sure to remove the cover lining. Crushed peels by mortar casserole to granularity given.

Put them in the oven heat with the temperature 900 °C for a period of one hour to turn material into powder snow-white, largely due to the fact that eggshells consist of material calcium carbonate $CaCO_3$ and when heating disintegrate to subjects of carbon dioxide and calcium oxide (CO_2, CaO) and by the following equation :

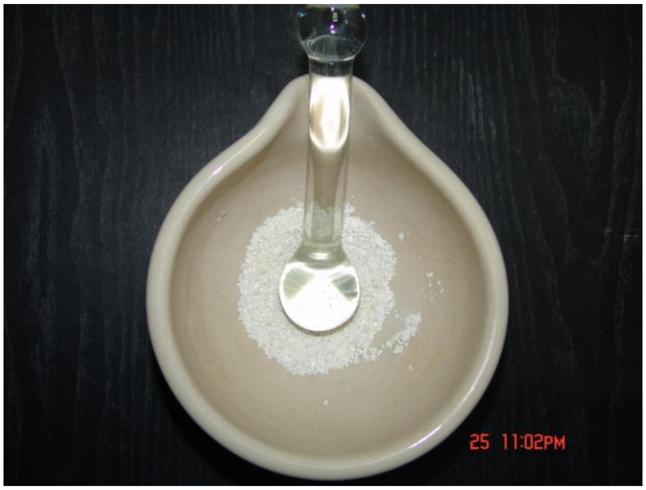
$$CaCO_3 \xrightarrow{\wedge} CaO + CO_2$$

Then by the slow addition of 0.6M H_3PO_4 (Phosphoric acid) to the aqueous (molar ratio) suspension of CaO under constant stirring, and formation of (HA) by equation

$$CaO + 6H_3PO_4 \xrightarrow{H2O} Ca_{10}(PO_4)_6(OH)_2$$

The output was cooled to room temperature(22±3) °C and then filtered using suppression Buchner with washing several times with distilled water, and then placed in an oven at a temperature of 110 °C for the purpose of drying and sterilization.

To obtain precipitate (HA) critical control of the pH of the reaction and concentration of the reactant is required. The final product was characterized for its crystallinity and phase behavior through instrumentation techniques (Figure 3.2) (HA) in jar(Ahmed, 2009).



25 11:02PM

Figure (3.2) Hydroxyapatite in jar

3.4.3 Heat Cured Acrylic Resin with and without Additive (HA) Samples Preparation

a. Acrylic resin specimen preparation

Acrylic resin specimens were prepared in a mould made by investing a hard elastic foil with specific dimensions to each test (Figure 3.3).

The dental stone was mixed with water in ratio; 100gm of powder was added to 23ml of distilled water according to the manufacturer instructions, with manual spatulation for 20-30 seconds. Gentle vibration by electrical vibrator was used for 1 minute to get rid of air bubbles, then mixture was poured into lower half of flask. Elastic foil was lubricated by using Vaseline before investing into dental stone which then allowed setting for 1 hr before pouring a second layer of dental stone. After setting, the stone surface was coated with Isodent separating medium; then the upper half of the flask was seated with dental stone and left for another 1 hr (Craig *et al.*, 1995).

Powder (polymer) and liquid (monomer) of heat cured acrylic resin have been mixed together in a dry clean glass jar 2.2 gm: 1ml by volume (according to the manufacturer instructions) for the control gp.

While the experimental groups of heat cured acrylic resin with additive have been prepared by mixing powder (polymer) (22gm polymer with 0.44gm hydroxyapatite for 2% and 8.8gm polymer with 0.44gm hydroxyapatite for 5%) with liquid consisted of together in glass jar 2.2gm:1ml by volume, after reaching dough stage, the mixture has been inserted into prepared mould

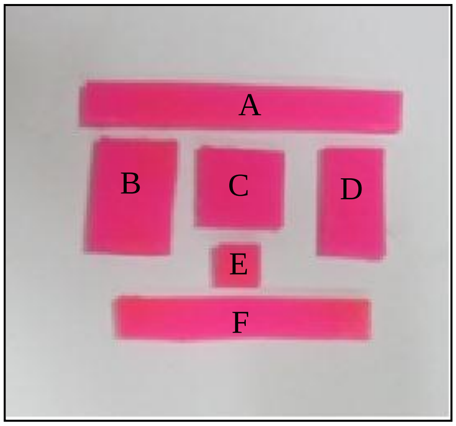


Figure (3.3) Hard Elastic Foil Moulds Used in the Preparation of the Samples. A. Tensile strength test, B. Indentation hardness test, C. Residual monomer test, D. FTIR test, E. Water sorption and solubility test, F. Transverse strength test.

Two-steps packing procedure has been used; over filling the dental stone mould by acrylic dough, then the cellophane paper has been placed above the dough for trial packing. The flask has been placed under press (800-2000 pound), and then opened, and access acrylic resin has been removed by sharp wax knife then left for 45 minutes before curing (Craig *et al*, 1996).

The acrylic resin has been cured in water bath. Put the flask in water bath at 20°C, elevate temperature until it reaches 100°C then remaining for 30 minutes and the flask was left a side for slow bench cooling before opening (according to manufacturer instruction).

The flasks were left for bench cooling at room temperature; the samples have been removed, carved with engine stone bur, polished with pumice, adjusted and incubated in distilled water at 37 ±1°C 48 hours for conditioning before testing (ADA specification No.12, 1975).

b. Relining sample preparation

A thickness of 1 mm relining material 1:1.5 mm of denture base was choosed in this study as 1mm of reduction of denture base before relining was taken by many authors (Ramzi, 2005) as shown in (Figure 3.4).

The samples were prepared by placing hard elastic foil with 1.8 mm thickness for indentation hardness and residual monomer test, 1.5 mm thickness for transverse strength, dimensional accuracy test and 2mm for water sorption and solubility test the samples were cut by using a sharp cutter to the desired length and width specified for each test.

The acrylic specimens prepared previously represent the denture base to be relined, the samples were placed in moulds with 2.5 mm thickness for transverse strength and moulds of 3 mm thickness for indentation hardness, dimensional accuracy test, tensile strength test and residual monomer test (from the control group preparation) and 4mm for water sorption and solubility test the polished surface faced downward and the tissue surface faced upward.

The acrylic polymer powder mixed with hydroxyapatite as mentioned in section (3.4.3.a) then mixed with monomer, the mixture was left till it reach dough stage, all of the samples were surface treated by application of methyl methacrylate monomer by using a fine brush for 180 sec. (Valittu *et al.*,1994; Arima *et al.*, 1996 ; Vergani *et al.,* 2000; Hasan, 2002).

The acrylic dough was applied over the tissue surface of the samples, then packing, curing, deflasking were processed then the samples were immersed in distilled water for 48 hour before testing.

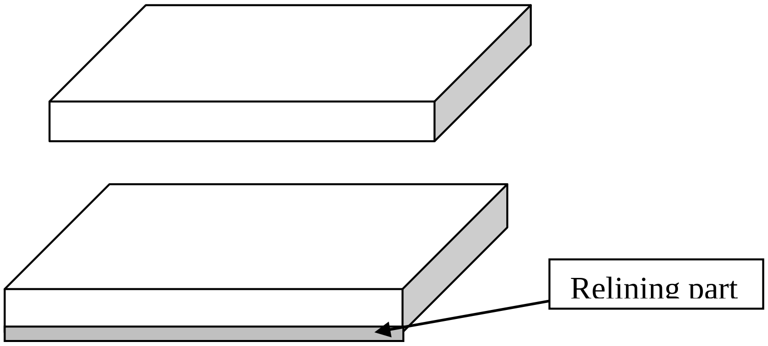


Figure (3.4)Diagram Showing the denture base with and without relining

3.5. Tests Used in this Study

3.5.1. Biocompatibility test

Twelve samples were prepared,4 samples of heat cured acrylic resin with 2% hydroxyapatite, 4 samples of heat cured acrylic resin with 5% hydroxyapatite, and the other four as a control with dimension of 5x2x2.5mm (length, width and thickness) respectively.

1. Condition of animal

Four local bred male rabbits, 4-6 months old with an average weight 1.25-1.35 Kg weight were used, the animals were housed in an animal house in the College of Dentistry University of Mosul, the animal were fed a normal diet of vegetables (2 times daily) and tap water according to (AL-Saigh , 2007).

2. Description of the experimental procedure :

a. General anesthesia:

An intra muscular injection of a mixture of general anasthesia ketamine hydrochloride (40 mg /kg) (katazing, 1998), and 0.3 ml xylazine (4 mg /kg) as a sedative, analgesic drug (Jones *et al*., 1978). Complete anesthesia had been obtained within 5 minute, this dose kept the animal anesthetized for about 40 minutes.

b. Implant procedure

The anesthetized animal was laid on it is abdomen on the operation board. The fur was shaved in 2 areas of the rabbits mandible one on right side and one on other side (the first rabbit in right side the control sample is placed and on the left side the 2% hydroxyapatite sample is placed and the second rabbit on right side the control sample is placed and on left side the 5% hydroxyapatite sample is placed).Then the shaved areas of the skin were disinfected using 5% hibitane. using no. 15 detectable blade on a scalpel handle , a small longitudinal incision (about 5mm) was made in the skin of each area, a pocket was created in the subcutaneous layer (by using a blunt dissection) to accommodate the implant.

The samples were applied immediately into created pocket, the skin were sutured with one stitch 3.0 black suture (Fig3.5, and 3.6).

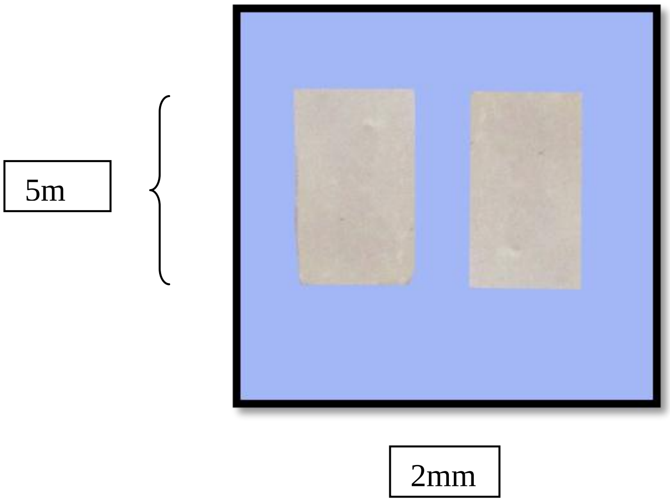
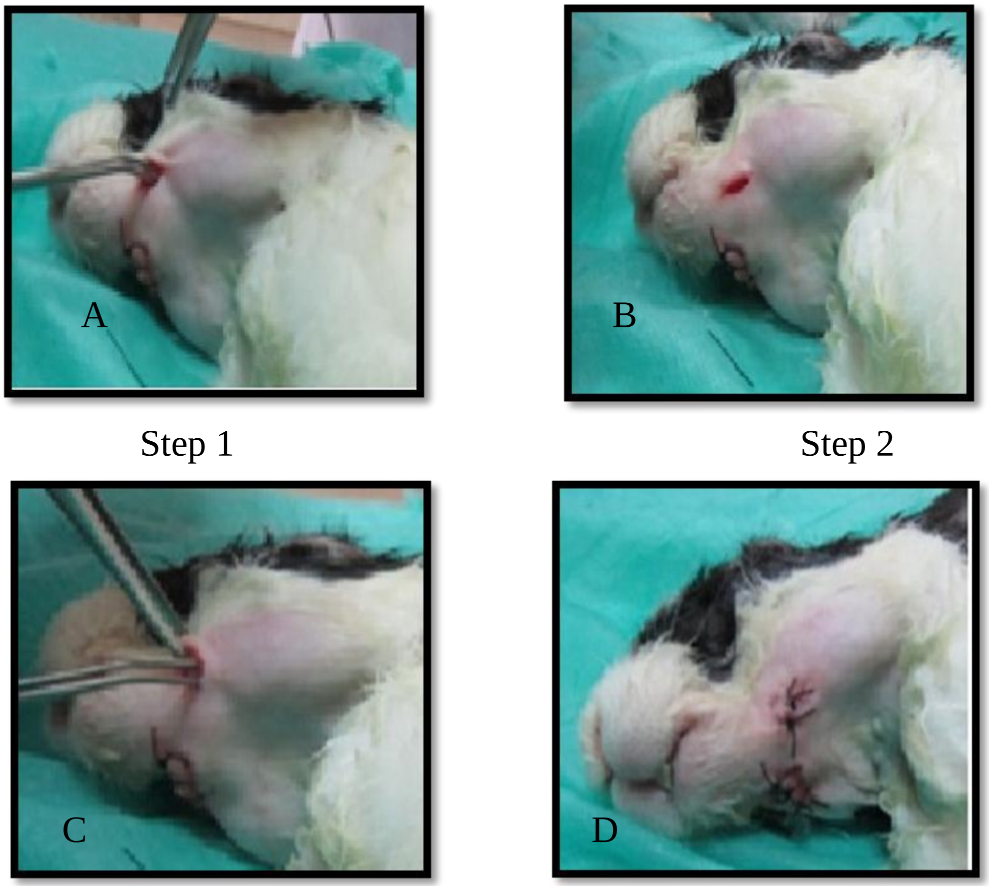


Figure **(3.5)** picture of implant samples



Step 1

Step 2

Step3

Step 4

Figure (3.6) Steps of samples implantation procedure

c. Post operative care

Immediately after the operation, a mixture veterinary antibiotics containing (0.5g) oxytetracycline had been administered intra muscularly in the muscle of the rabbit (Jones *et al* .,1978). Same dose of oxytetracycline were repeated every 24 hours for five days. During this period the animals was isolated from other animals as they will try to harm the animal or remove the suture.

d. Preparation of the specimens for Histopathalogical examination

After 30 days of implantation the animal were sacrificed, the biopsy specimen of bone had been excised in the following manner :

The sacrificed animal was laid on its abdomen then the implantation sites were examined grossly, the implantation and control sites were excised from the skin and kept in 10% formalin for 48 hours, processed in alcohol and xylol embedded in paraffin wax, sectioned at 5 micron thickness, stained with haematoxylin and eosin and examined under light microscope according to (Luna, 1988).

e. Histopathalogical examination

The Histopathalogical test done by two specialist Doctor (every slide examined by two examiner) one in Department of pathology in the College of Veterinary Medicine, and other in Department of pathology in College of Dentistry.

3.5.2 FTIR Test

The degree of conversion of the materials under investigation was determined by Fourier transform infrared spectroscopy (FTIR) carried by Tensor 27 FTIR spectrophotometer (3.7) in university of Mosul. The samples of heat polymerizing resin with dimension of 10x4x4(±0.03)mm (length, width and thickness) respectively were prepared and divided into three groups [first group control samples without additive Five samples , second group with 2% (HA) Five samples, third group with 5% (HA) Five samples] after that the specimens were finished with stone bur and sand paper to remove any gypsum product on their surfaces then they polished and stored in distilled water at 37 (±1) °C for 48 hours (Urban *et al*., 2007) After 48 hours, the samples were removed from water and dried in air and then scraped using a sharp, clean and sterile wax knife to obtain powder of the polymerized samples. Then 300 mg of the sample powder was grinded finely, under anhydrous conditions, in an agate mortar. This powder is then thoroughly mixed with 100-200 mg of oven –dried, spectral-grade; 100-200 mesh potassium bromide powder. The mixture pellets is then mounted on a holder and placed in the sample beam of spectrometer (Parikh,1974).

Figure(3.7) Tensor 27 FTIR spectrophotometer

3.5.3. Microscopical Examination of Hydroxyapatite

Microscopical examination was done by using Taha indicator **(**which is a transparent fluid oil, used as a lubricant for sewing machine; chemically is one of the saturated cyclic hydrocarbons which is relatively inert, don’t react with most of common acids, bases or oxidizing or reducing agents) (Hart, 1987).

1. Mixing about 1mg of non milled Hydroxyapatite on glass slide with one drop of Taha indicator then covered with glass cover and examined with Motic digital microscope.
2. Mixing about 1mg of Hydroxyapatite after milling it on glass slide with one drop of Taha indicator then covered with glass cover and examined with Motic digital microscope.
3. Putting about 1mg of non milled (HA) on glass slide then examined with Motic digital microscope.
4. Putting 1mg of milled (HA) on glass slide then examined with Motic digital microscope (Hart, 1987).

3.5.4. Indentation Hardness Test

Total samples were prepared as in section (3.4.3) and with dimension 30xl5x3±0.03 mm (length, width, and thickness) respectively. The samples stored in a distilled water at 37°C for 48 hours before testing, and divided into 5 groups.

The samples surfaces were tested for hardness at five different locations then the mean is taken for each sample (Issac, 1992).

The test was done by using Rockwell hardness tester, equipped with an indenter in the form of round steel ball of 1/4 inch in diameter.

The samples were first subjected to fixed minor load 60 Kg (according to the instruction machine) (Figure 3.8), in University of Mosul.

Figure (3.8) Indentation Hardness Machine

3.5.5. Transverse Strength Test

Total samples were prepared with dimensions of 65x10x2.5±0.03mm (length, width and thickness) respectively were prepared as in section (3.4.3), according to ADA specification no. 12. The samples stored in a distilled water at 37°C for 48 hours, and divided into 5 groups.

The test was applied by using a 3 points bending on an Instron testing machine. The device was supplied with a central loading plunger and two supports, with polished cylindrical surfaces of 3.2 mm in diameter and 50 mm between supports. The supports should be parallel to each other and perpendicular to the central line (Al-Nema, 2004). The tests were carried out with cross head speed of 5mm/min.

The test samples held at each end of the two supports, and the loading plunger placed mid way between the supports. The samples were deflected until fracture occurred (Figure3.9), in University of Mosul.

The transverse strength were calculated using the following equation:

$S=3Pl/ 2bd^2$

S= transverse strength (N/mm^2)   b= width of specimen (mm)

d= depth of specimen (mm)

I= distance between supports (mm)

P= maximum force exerted on specimen (N) (Criag and ward, 1997).

Figure (3.9) Transverse testing machine.

3.5.6 Water Sorption and Solubility Test

Total samples were prepared with dimensions of 10 x12x4±0.2mm (length, width and thickness) respectively (Podgorski, 2010).

The samples were dried in oven at 37 °C until their weight was constant, and the result was recorded as m_1. The specimens were then immersed in distilled water and maintained at 37 °C for a week. After this time, the samples were removed, blotted to remove surface water, dried in air for 15 second, and weighed. The result was recorded as (m_2). the specimens were placed in the desiccators that contained an hydrous calcium chloride and dried at 37 °C until a final constant mass was obtained (m_3) The volumes of the specimens volume (v) were also measured (Podgorski, 2010).

To calculate the water sorption and solubility the following equations were used:

Water sorption $=m_2-m_1/v$

Water solubility $=m_2-m_3/v$

3.5.7. Tensile Bonding Test

Total samples were prepared with dimensions of 90×10×3±0.03mm (length, width and thickness) respectively were prepared representing Five samples for the control group then for relined group (five samples for 2% HA and. Five samples for 5% HA). A metal spacer was placed at the center of the mould with dimensions of 10×10×3 mm, then packing and curing were done, after curing the two parts of the sample were removed and stored in distilled water at 37^{o}C for 24 hr. before relining. Then the two acrylic parts of each sample were placed back into the moulds, surface treatment was done at the two surfaces facing the space made by metal spacer. After that, the relining material with additive (2% & or 5% HA) were packed into the space then curing were proceeded (Ramzi, 2005) as shown in (Figure 3.10).The same procedure is done for control samples but without adding (HA) to relined material. The samples were conditioned at 37^{o}C for 48 hr. in distilled water before testing (Salem *et al.,* 1990).

The samples were grasped by two arms of the Gunt universal testing machine (Figure 3.11) in university of Irbil, and amount of force applied was 1 Newton per second continuous tension force until fracture of the sample occurred by The result were record from a special program on computer of tensile machine for each sample, the force of failure was recorded in Newton (N) and the true tensile bond strength value was calculated by the following formula:

Tensile bonding strength = force of failure / cross sectional area

(Ozkan *et al*., 2003)

The mode of failure was evaluated by microscope and characterized as:

a. Adhesive failure refers to total separation at the inter face between the relining material and denture base.

b. Cohesive failure refers to fracture within the relining of denture base.
c. Mixed failure refers to both.

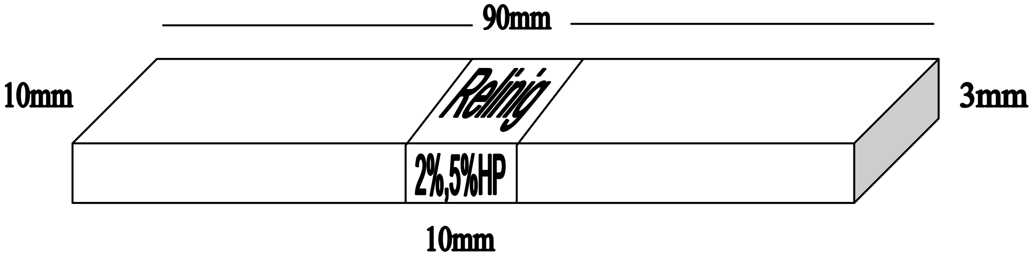


Figure (3.10) Sample of Tensile Bonding Test

Figure (3.11): Gunt Universal testing machine

3.5.8. Dimensional Accuracy test

Total samples were prepared with dimensions of 20*20*3±0.03mm (length, width, and thickness respectively) as in section (3.4.3) according to (ADA specification No.12 1975). Measurements on three dimensions were done by using electronic digital caliper accuracy of 0.01mm. Marks, were placed into the samples to allow measurement length, width, and thickness of sample.

The dimensional accuracy samples for heat cured acrylic resin with additives 2%,and 5%Hydrxyapatite compared with control group after 2 days immersion in distilled water.

3.5.9. Residual Monomer Test

Total samples were prepared with dimensions of 20×20×3±0.03mm (length, width and thickness) respectively, for all relined samples with additive 2%, and 5% Hydroxyapatite, the ratio of relining to denture base was 1.2:1.8mm.

Each sample was introduced in a sealed glass flask containing 10 ml. of distilled water at 37°C . At appropriate time intervals (1st,2nd,3rd ,4th , 5th , 6th , and 7th days), the supernatants were removed and replaced by 10 ml of fresh distilled water. The time dependence of the monomer concentration was followed by monitoring the amount of monomer present in the supernatant medium using a (CECIL, 2000) ultraviolet-visible spectrophotometer (λ=254 nm). (Azzarri *et al.*, 2003).

A linear calibration curve of methyl methacrylate(MMA)concentration as a function of absorbance at 254 nm (Figure 3.12). was obtained using MMA standard aqueous solutions in the range 0.025-0.5 mg/ml.

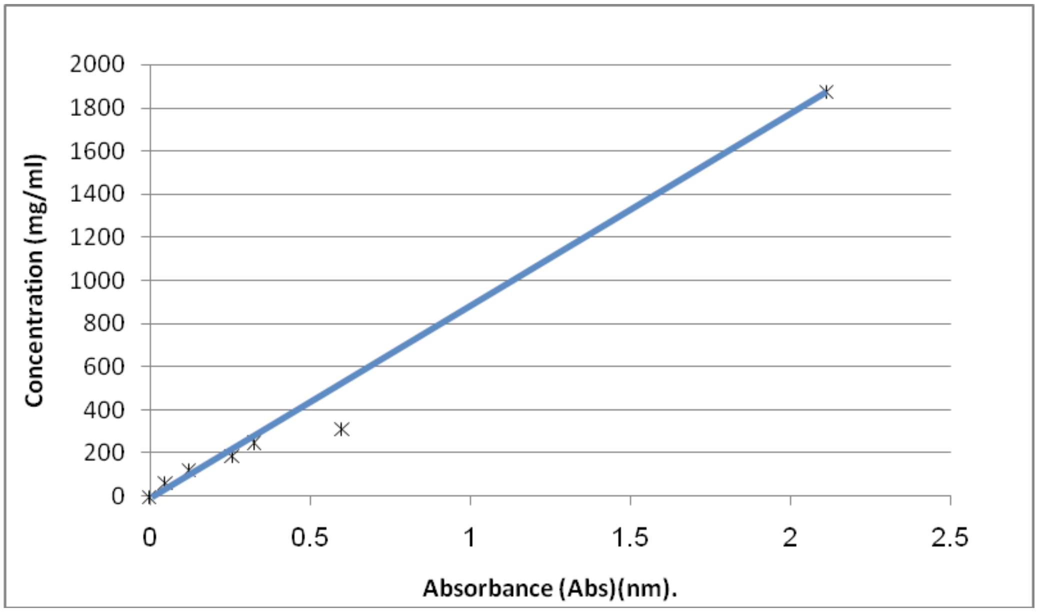


Figure (3.12): calibration curve

The results were expressed as a percent of released residual monomer mass with respect to the weight of the specimen (Azzarri *et al.,* 2003).

3.6 Statistical Analysis

The following statistical methods were used to analyze and assess the results via SPSS V. 11.5 for Windows:

1) ANOVA and Duncan multiple range test were used. The statistical results were considered significant at $p \leq 0.05$.

2) Paired T-test for measuring volumetric changes.

CHAPTER FOUR

Results

4.1 Biocompatibility Test

Biocompatibility test shown new bone formation in the rabbits after 30 days of implantation for both 2% and 5% Hydroxyapatite samples as shown in Figure (4.1).

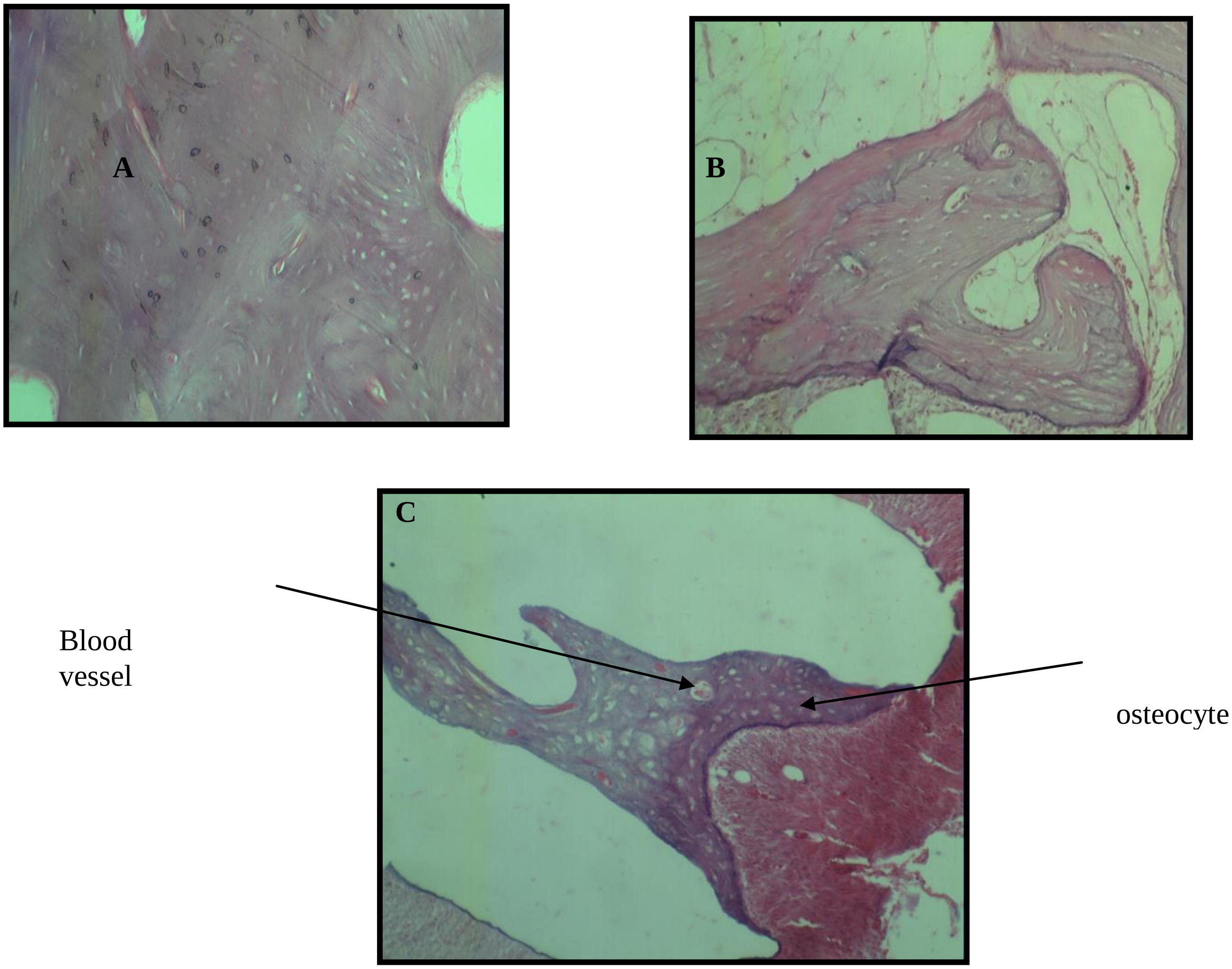
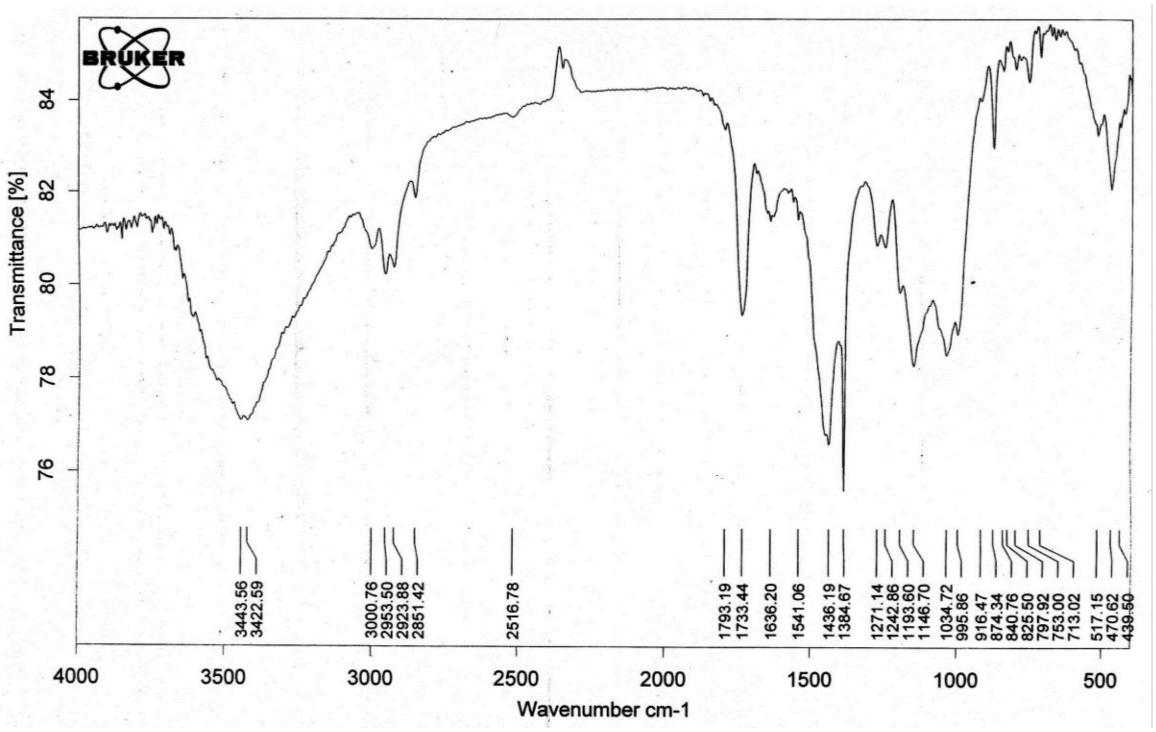


Figure (4.1)A. controlled bone(without additives), B. new bone in sample with(2% Hydroxyapatite), C. new bone in sample with(5%Hydroxyapatite).

The new bone characterized by pink in color, more spongy in its texture, more blood vessels and more osteocytes content. That is differe from old bone which is characterized by more compact in its texture, darker in color and less blood vessels and less osteocytes content, the results show no differences between 2% and 5% samples containing Hydroxyapatite, the two samples show the same effect on the bone.

4.2 FTIR test

In the IR charts two important absorbance peaks appeared (the absorbance of the C=C band from the methacrylate group which appear around 1640 cm^{-1} and the absorbance peak of the C=O from the ester group appear around 1720 cm^{-1}) as shown in (Figure 4.2-4.4).



Figure(4.2) :Control acrylic resin only

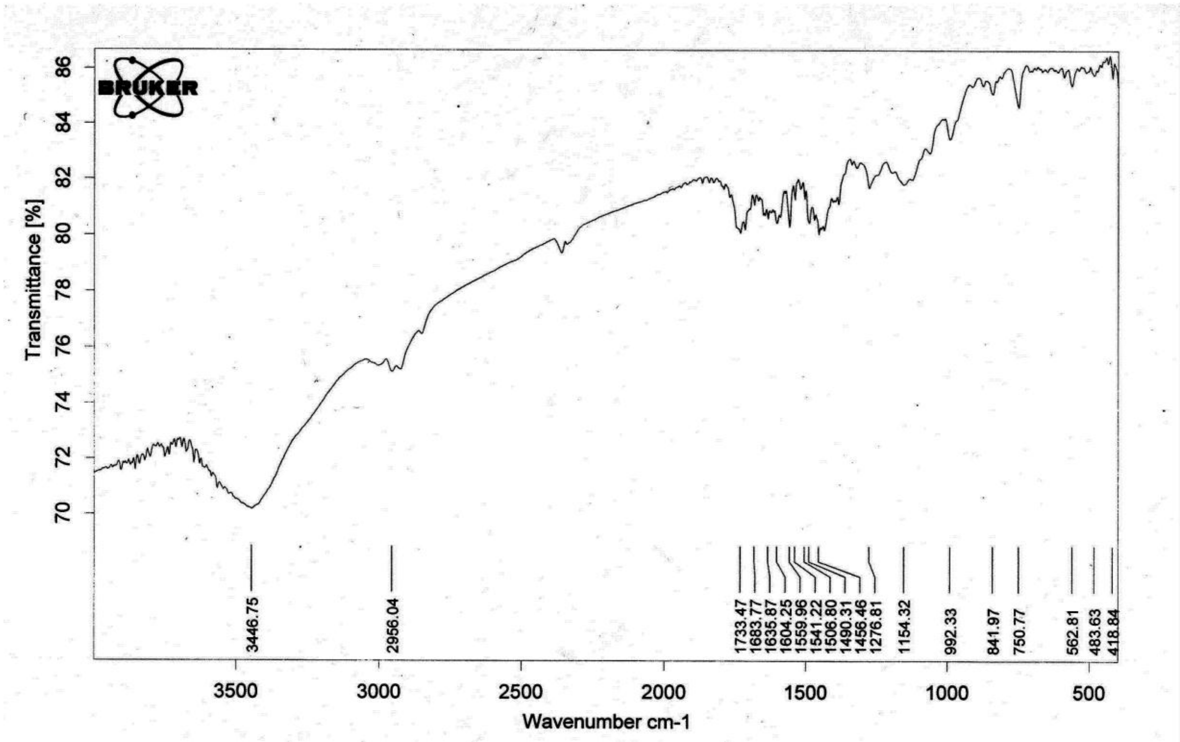
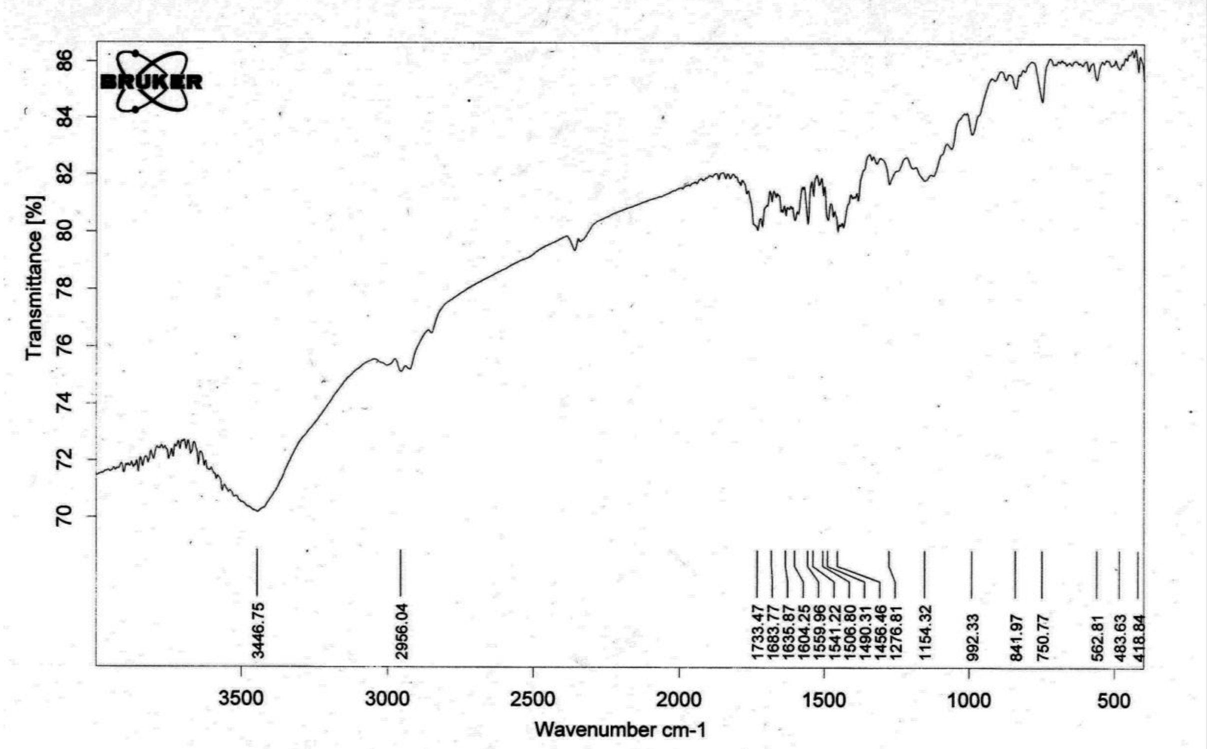


Figure (4.3) : Acrylic resin with 2% hydroxyapatite



Figure(4.4): Acrylic resin with 5% hydroxyapatite

4.3. Microscopical Examination of Prepared Hydroxyapatite

This test show that the (HA) particle appear as crystalline in both milled and non milled (HA) as shown in Figure (4.5).

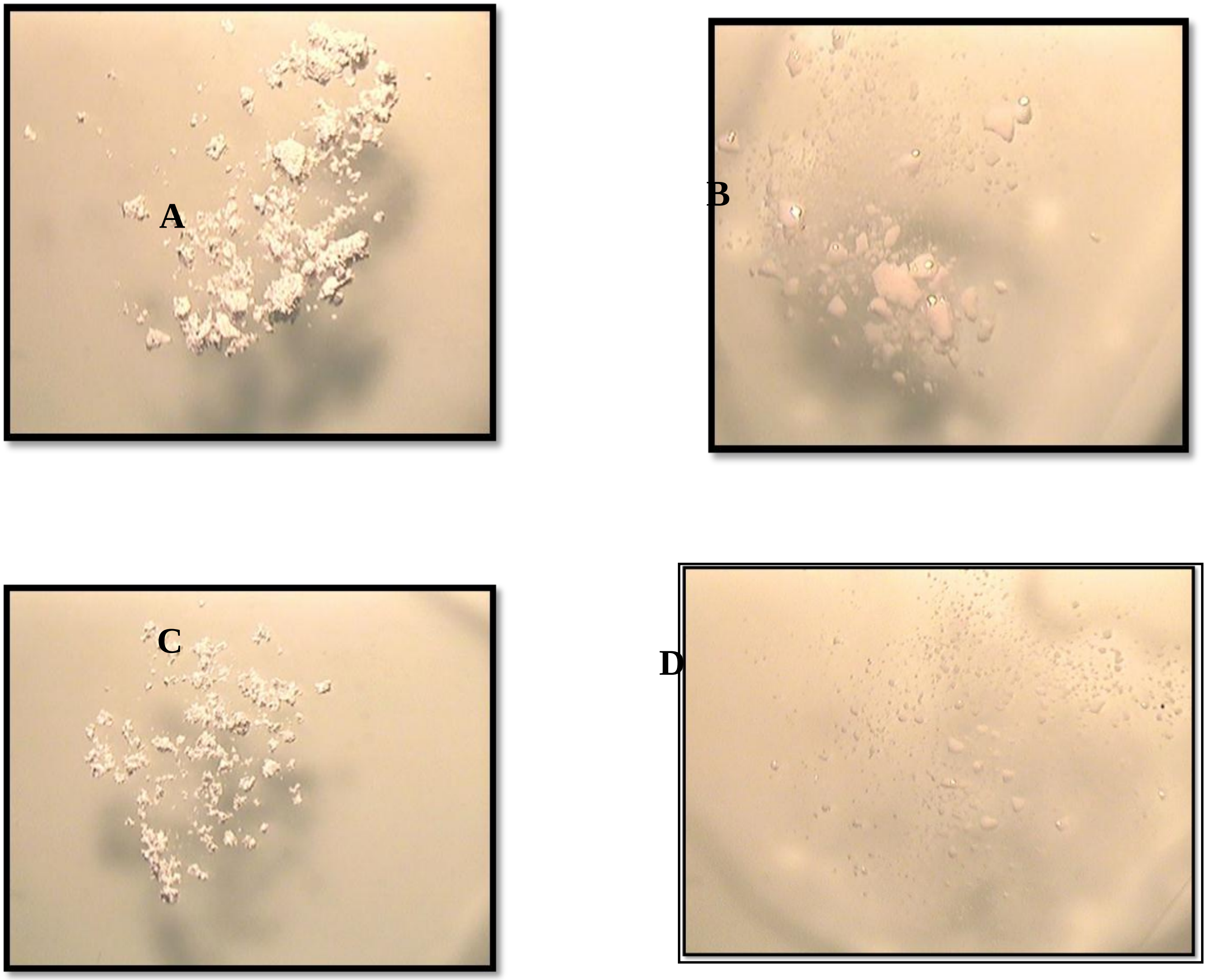


Figure (4.5): A. (HA) powder before milling without taha indicator, B. (HA) powder before milling with taha indicator, C. (HA) powder after milling without taha indicator, D. (HA) powder after milling with taha indicator

4.4 Hardness test

Indentation Hardness of five different groups of Heat Cured Acrylic Resin (HCAR), the first group (HCAR) only, 2nd group (HCAR) mixed with 2% Hydroxyapatite (HA) , 3rd group (HCAR) relined with 2% Hydroxyapatite (HA), 4th group (HCAR) mixed with 5% Hydroxyapatite (HA) and 5th group (HCAR) relined with 5%(HA) was evaluated. Indentation hardness mean and standard deviation were shown in Figure (4.6).

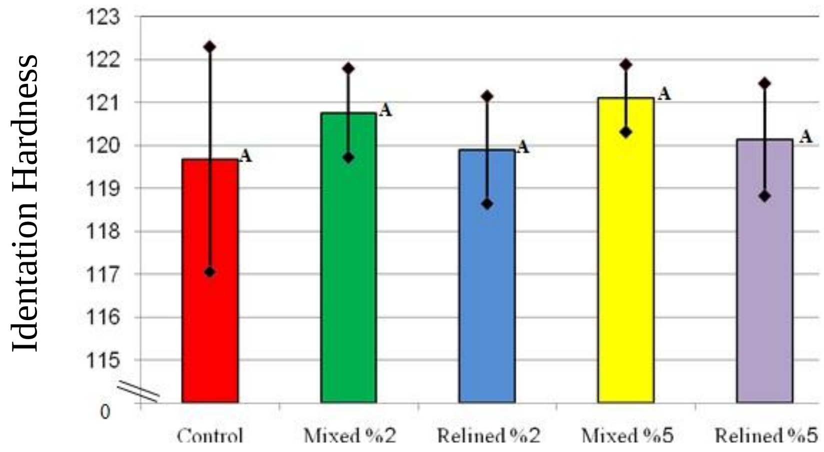


Figure (4.6) Hardness mean ,standard deviation and Duncan's multiple range test.

different Letters means significant differences for Duncan's multiple range test.

One way ANOVA of Indentation Hardness showed no significant difference between tested groups at $P \leq 0.05$ (Table 4.1).

Duncan's multiple range of Indentation Hardness for the tested groups were shown in Figure (4.6)

Table (4.1) one way ANOVA of Indentation Hardness of tested groups

	Sum of Squares	**df**	**Mean Square**	**F**	**p-value**
Between Groups	7.102	4	1.775	.758	.565
Within Groups	46.872	20	2.344		
Total	53.974	24			

4.5 Transverse Strength:

Transverse strength of five of different groups of Heat Cured Acrylic Resin (HCAR), shown increase in transverse strength in (HCAR) mixed with 5% (HA) and (HCAR) mixed with 2% (HA) then followed by (HCAR) relined with 5%(HA) and (HCAR) relined with 2% (HA). Transverse Strength mean and standard deviation were shown in Figure (4.7).

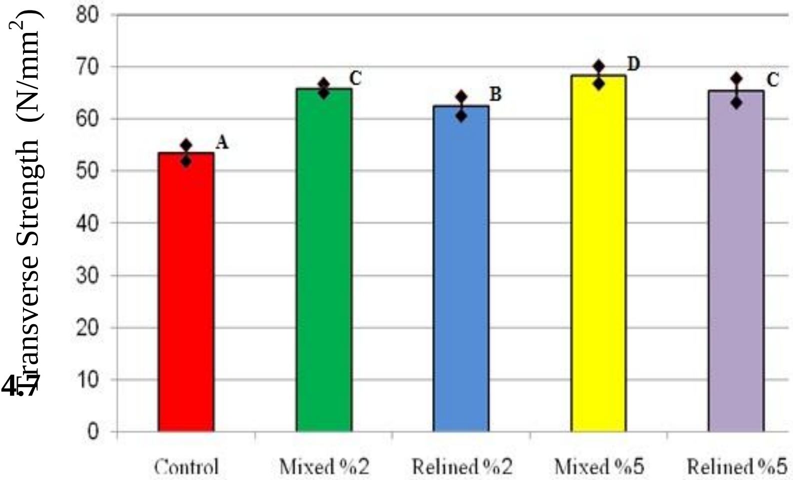


Figure (4.7 s multiple range

different Letters means significant differences for Duncan's multiple range test.

One way ANOVA of Transverse strength showed significant difference between tested groups at $P \leq 0.05$ (Table 4.2).

Duncan's multiple range of Transverse strength for the tested groups were shown in Figure (4.7)

Table (4.2) one way ANOVA of Transverse strength of tested groups

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	676.240	4	169.060	58.701	.000
Within Groups	57.600	20	2.880		
Total	733.840	24			

4.6 Water sorption test

Water sorption of five of different groups of Heat Cured Acrylic Resin (HCAR), the first group (HCAR) only, 2nd group (HCAR) mixed with 2% Hydroxyapatite (HA), 3rd group (HCAR) relined with 2% Hydroxyapatite (HA), 4th group (HCAR) mixed with 5% Hydroxyapatite (HA) and 5th group (HCAR) relined with 5%(HA) was evaluated. Water sorption mean and standard deviation were shown in Figure (4.8).

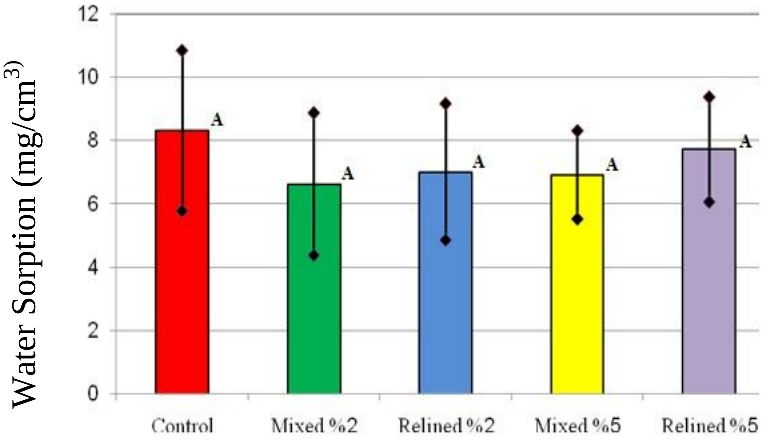


Figure (4.8) Water sorption mean ,standard deviation and Duncan's multiple range test.

different Letters means significant differences for Duncan's multiple range test.

One way ANOVA of Water sorption showed no significant difference between tested groups at $P \leq 0.05$ (Table 4.3).

Duncan's multiple range of Water sorption for the tested groups were shown in Figure (4.8)

Table (4.3) one way ANOVA of Water sorption of tested groups

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	9.418	4	2.355	.564	.691
Within Groups	83.432	20	4.172		
Total	92.851	24			

4.7. Water Solubility

Water Solubility of five of different groups of Heat Cured Acrylic Resin (HCAR), the first group (HCAR) only, 2nd group (HCAR) mixed with 2% Hydroxyapatite (HA), 3rd group (HCAR) relined with 2% Hydroxyapatite (HA), 4th group (HCAR) mixed with 5% Hydroxyapatite (HA) and 5th group (HCAR) relined with 5%(HA) was evaluated. Water Solubility mean and standard deviation were shown in Figure(4.9).

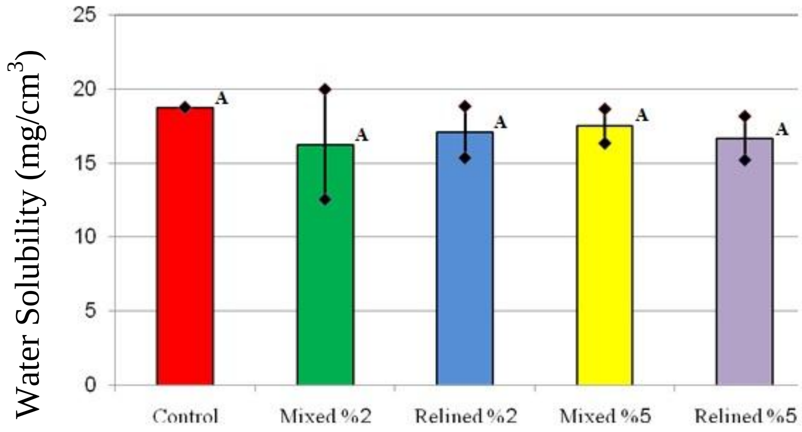


Figure (4.9) Water Solubility mean ,standard deviation and Duncans multiple range test.

different Letters means significant differences for Duncan's multiple range test.

One way ANOVA of Water Solubility showed no significant difference between tested groups at P≤ 0.05 (Table 4.4).

Duncan's multiple range of Water Solubility for the tested groups were shown in Figure (4.9).

Table (4.4) one way ANOVA of Water Solubility of tested groups

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	18.355	4	4.589	1.124	.373
Within Groups	81.673	20	4.084		
Total	100.029	24			

4.8 Tensile Strength Test

Tensile strength of three of different groups of Heat Cured Acrylic Resin (HCAR), the first group (HCAR) only, 2nd group (HCAR) relined with 2% Hydroxyapatite (HA) and 3rd group (HCAR) relined with 5% Hydroxyapatite (HA), was evaluated. Tensile Strength mean and standard deviation were shown in Figure (4.10).

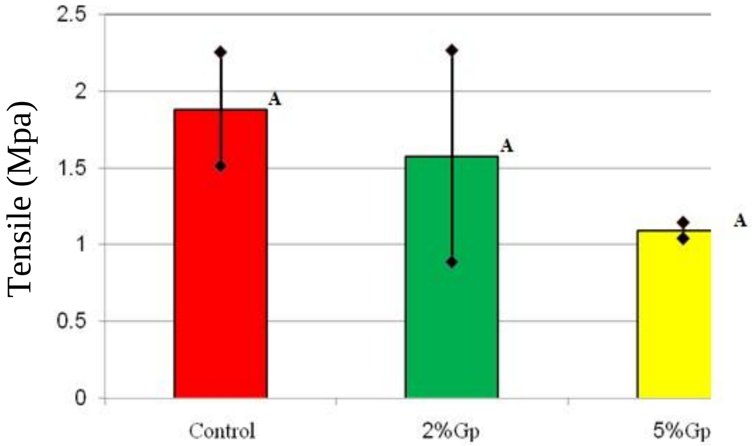


Figure (4.10) Tensile strength mean ,standard deviation and Duncan's multiple range test. *different Letters means significant differences for Duncan's multiple range test*.

One way ANOVA of Tensile strength showed no significant difference between tested groups at $P \leq 0.05$ (Table4.5).

Duncan's multiple range of tensile strength for the tested groups were shown in Figure (4.10).

Table (4.5) one way ANOVA of Tensile strength of tested groups

	Sum of Squares	**df**	**Mean Square**	**F**	**p-value**
Between Groups	1.577	2	.789	3.868	.051
Within Groups	2.447	12	.204		
Total	4.024	14			

Mode of failure

Examination of the samples by Motic digital microscope show :

1. Controled samples show 100% cohesive failure as shown in Figure (4.6).
2. All relined samples showed 100% cohesive adhesive failure in both 2% and 5% (HA) adding to acrylic resin as shown in Figure (4.11).

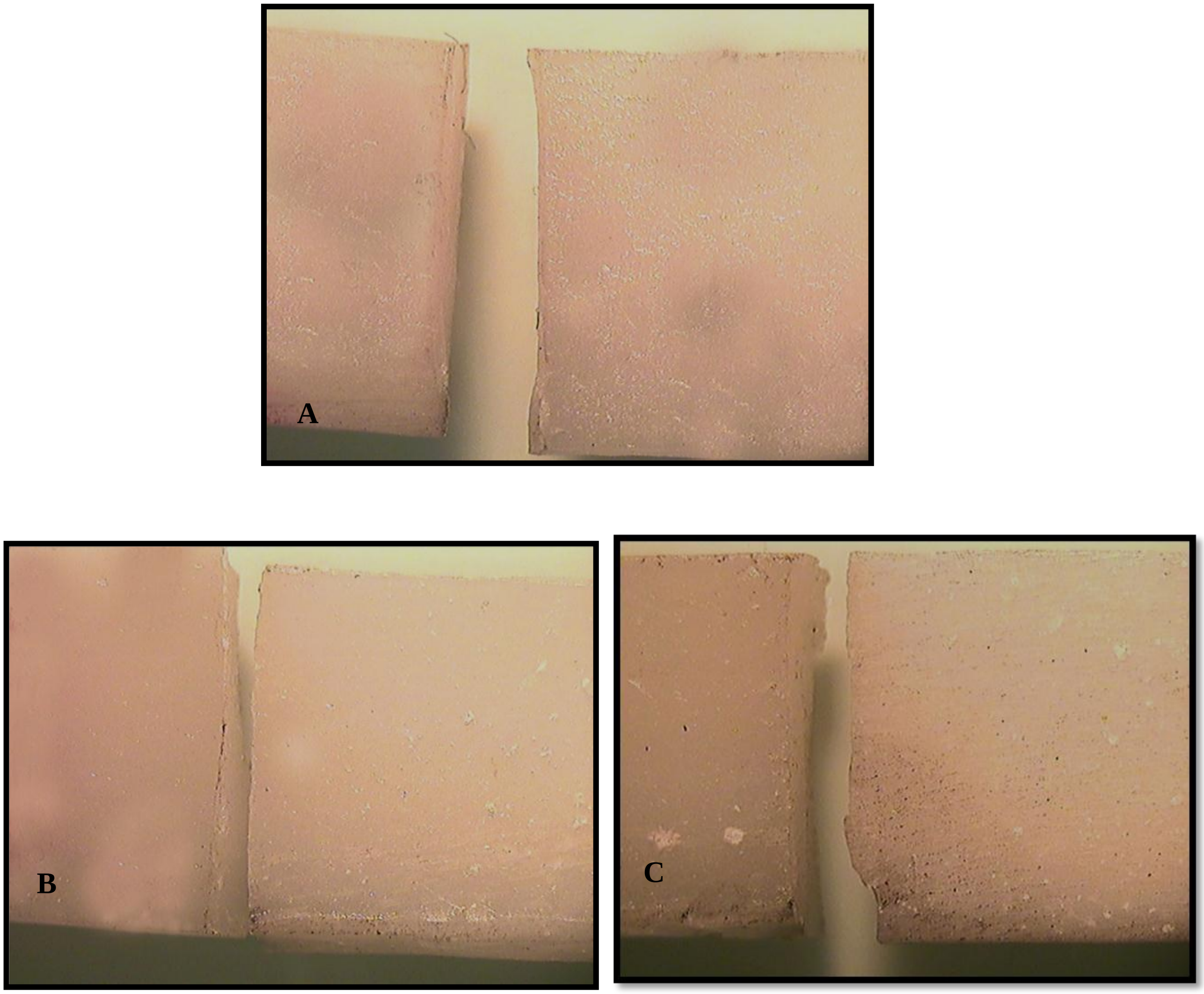


Figure (4.11) :A. show the fail in control sample, B. show the fail in 2% (HA), C. show the fail in 5% (HA).

4.9 Dimensional Accuracy Test

Paired T test for Dimensional accuracy for five groups heat cured acrylic (HCAR), first group (HCAR) only, (HCAR) mixed with 2% (HA), (HCAR) relined with 2% (HA), (HCAR) mixed with 5% (HA), (HCAR) relined with 5% (HA) showed no significant at $P \leq 0.05$ (Table 4.6).

Table(4.6):T-test of Dimensional accuracy between all groups

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Before	1200.0000	5	.00000	.00000
	After	1502.7000	5	293.76343	131.37500

Paired Samples Test

		t	df	p-value
Pair 1	Before - After	-2.304	4	.083

4.10. Residual Monomer

Residual monomer of five of different groups of Heat Cured Acrylic Resin (HCAR), the first group (HCAR) only, 2nd group (HCAR) relined with 2% Hydroxyapatite (HA), 3rd group (HCAR) relined with 5%(HA) show release of residual monomer up to third day and no release in fourth and fifth day, but 4th group (HCAR) mixed with 2% Hydroxy apatite (HA) and 5th group (HCAR) mixed with 5% Hydroxyappetite (HA) the result show no residual monomer release after adding Hydroxyapatite as shown in Figure (4.12).

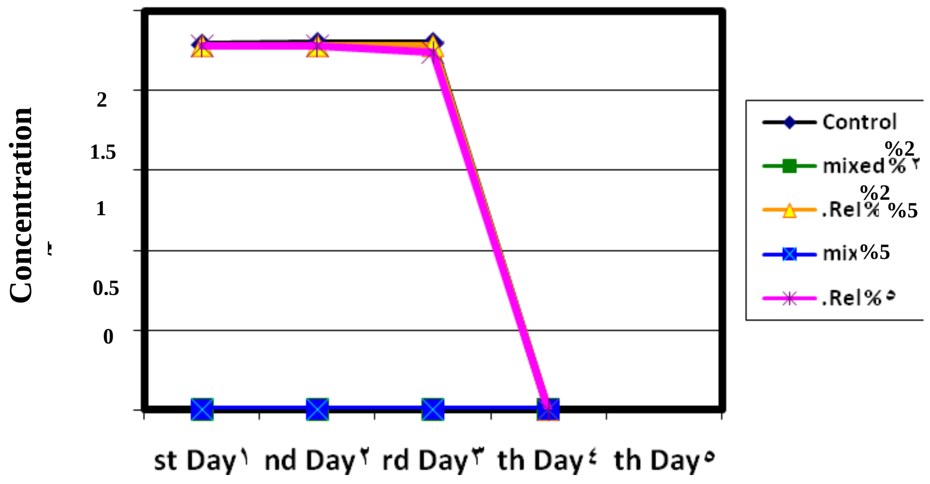


Figure (4.12): concentration of residual monomer release

1St Day 2nd Day 3rd Day 4th Day 5th Day

CHAPTER FIVE

Discussion

There is no previous study similar to our study, so there is no previous research to support this result.

5.1. Biocompatibility Test

In the experimental groups, the body reaction to the implanted show good response since there was no clinical signs of inflammation. As hydroxyapatite is synthesized as a dublicate of minerals naturally present in bones and teeth, hydroxyapatite usually shows minimal tissue reaction (Kandaswamy *et al,* 2000). The presence of osteoblasts and fibroblast cells in the histological slides may directly indicate that experimental material allow cell proliferation without a cytotoxic effect, and they are biocompatible with cells.

The histopathological examination showed more new bone trabeculae formation in the (HA) than control, a finding that agrees with study of AL-Khafaji, (2001) which showed active bone formation started after one month of implantation. This finding may be due to more late neovascularization and release of calcium from the graft into surrounding tissue.

5.2. FTIR Test

In the IR charts, two important absorbance peaks appeared (the absorbance of the C=C band from the methacrylate group which appear around 1640 cm^{-1} and the absorbance peak of the C=O from the ester group appear around 1720 cm^{-1} .

Fourier transform infrared spectroscopy (FTIR) is a widely used analytical technique that is routinely applied to the characterization of biomaterials. Hydroxyl stretch is observed at 3569 cm~1 in the spectra of synthetic commercial hydroxyapatite.

The IR spectrum of raw HAP powder. The broad peak ranging from 3300-3600 cm^{-1} can be explained owing to O-H group stretch vibration. The band at 1450 cm^{-1} is assigned to the CO_3^{2-} stretching. An intense PO_4^{3-} peak appeared at 1048 cm^{-1}. Additional phosphate group bands are found in the region 963, 875, 633 and 472 cm^{-1}., a sharp peak of OH stretch vibration band appeared at 3570 cm^{-1} and a less intense CO^{3-} peak appeared at 1450 cm^{-1}.

An intense PO_4^{3-} peak appeared at 1048 cm^{-1}. The additional phosphate peaks are found in the region 1110, 975, 600 and 470 cm^{-1}.

The C-O-C peak at 1254, 1200 cm^{-1} and an intense phosphate peak is found is found at 1040 cm^{-1}. The additional phosphate group bands at 810 cm^{-1} and 757 cm^{-1}. In this spectrum, there is no peak at 2270 cm^{-1}.

The FT-IR spectrum of PMMA (Figure 4.3) indicates the details of functional groups present in the synthesized PMMA. A sharp intense peak at 1731 cm^{-1} appeared due to the presence of ester carbonyl group stretching vibration. The broad peak ranging form $1260\text{-}1000^{-1}$ can be explained owing to the C-O (ester bond) stretching vibration. The broad band from 950-650 cm^{-1} is due to the bending of C-H. The broad peak ranging from 3100-2900 cm^{-1} is due to the presence of stretching vibration.

Figures(4.4 and 4.5) shows the IR spectrum of HA/PMMA . The band at 3000 cm^{-1} is assigned to CH_3 stretch vibration. A very strong band appeared at 1735 cm^{-1} belongs to the carbonyl group,. Band at 2940 cm^{-1} is from $–CH_2$ stretch vibration. The C-O-C peak at 1254, 1200 cm^{-1} and an intense phosphate peak is found at 1040 cm^{-1}. The additional phosphate group bands at 810 cm^{-1} and 757 cm^{-1}. The FT-IR spectra provide strong proof that the functionalized PMMA were actually grafted to(HA) through In the spectra, therefore, a PMMA spectrum is clearly recognizable (Balamurugan *et al* ;2004).

From the FTIR charts for control and heat cured acrylic resin after adding hydroxyapatite they showed there are no any change in the region of bands for C=C and C-C to polymer before and after addition of (HA) this indicate there is no chemical reaction occurred and this mean there are no any conversion factor happen but the additive minimize the residual monomer

The findings of this study were agreed with Misch, (1993) and Kanczler, (2008) who concluded that the living cells, primerly from tubercular region, may actually live and form an osteoid product However the blood supply and cell numbers greatly influence the result .

5.3. Microscopical Examination of Prepared Hydroxyapatite

The Hydroxyapatite practicles appeared crystalline in nature this agreed with study of (Murakami *et al*., 2007) who showed that the morphology of the particles was investigated by optical microscopy for both industrial and egg shell calcium carbonates. The images of industrial product and egg shell product indicate that these powders are preferably crystalline, and the size of the egg shell product is larger than industrial calcium carbonate.

5.4. Indentation Hardness Test

The indentation hardness result of heat cured acrylic resin (HCAR) and Duncan's multiple range tests of it presented in Figure (4.6), It showed that (HCAR) mixed with 5% Hydroxyappetite (HA) has theoroticaly highest indentation hardness followed by (HCAR) mixed with 2% (HA) followed by (HCAR) relined by 5%(HA), and followed by (HCAR) relined by 2%(HA) then lastly the control sample.

The higher surface hardness value can be attributed to the higher polymerization in heat cure acrylic resin, and the lesser hardness value can be due to incomplete polymerization ratio and presence of residual monomer in acrylic resin (Srividya *et al*., 2011).

This study agree with Abdulhamed and Mohammad, (2010) whom showed that the surface hardness increases can be due to the randomly distributed particles of a hard material into acrylic matrix.

5.5. Transverse Strength Test

The transverse strength result of heat cured acrylic resin (HCAR) and Duncan's multiple range tests of it presented in Figure (4.7). It showed that adding Hydroxyapatite to heat cured acrylic resin will significantly increase transverse strength in comparison with the un reinforced acrylic resin specimens. So, the transverse strength of (HCAR) mixed with (HA 2% and/or 5%) was more than the results of (HCAR) relined with (HA) with the same percentage and the (HCAR) without additive show to the least value.

This result agreed with the result of study of Vodjdani and Kalid, (2006) who show that the transverse strength of heat polymerized denture base resin was considerably enhanced by including either metal wires or glass fibers. It has long been hypothesized that the addition of synthetic fibers to the monomer-polymer mixture may strengthen the resultant acrylic resin and several different types of fibers have been investigated.

If fibers are to be used to strengthen a polymer material, optimal adhesion between the fibers and the polymer matrix is essential. Impregnation of reinforcing fibers with resin allows fibers to come into contact with the polymer matrix (Vallitu, 1995; Kanie *et a.,l* 2006).

5.6. Water Sorption and Solubility Test

The water sorption and solubility results of heat cured acrylic resin (HCAR) and Duncan's multiple range tests of it presented in Figure (4.8 and4.9) which showed no significant differences between tested groups.

Decrease in the values of water solubility of the specimens theoroticaly containing Hydroxyapatite (HA) with different percentages, these decreases can be attributed to the fact that (HA) insoluble in water so that the addition of (HA) to the mass of the specimens which act as impurities and their presence will lead to reduction in the solubility of acrylic resin.

This study come in agree with Abdulhamed and Mohammad , (2010) who show that high solubility is not a favorable characteristic for denture materials. The denture base resins are insoluble in water and in most fluids that it's exposed to the resin in the oral cavity. Any observed loss of weight of the resin is a measure of the specimens solubility.

This agree with Kazanji *et al*., (1988) who show that the rate at which the materials absorbed water or lost soluble components varied considerably with the type of material, the amount of the plasticizer or filler content and the solution in which they were immersed.

Sorption can be related to the polarity of acrylic resin(due to unsaturated bond) (Philips,1991). While, solubility can be related to the leach of soluble material that present in an acrylic resin (initiator, plasticizer, residual monomer) (Takashi *et al*., 1995).

This study come in agree with Ana *et al*., (1998) who show that the addition of chemical materials caused alteration in molecular resin chain (modify the magnitude of the polymeric gaps) and consequently alter water sorption and solubility, also agree with Mienttinen and Vallitu, (1997) who show that water sorption and solubility depended on homogeneity of the material.

Also the study agree with Craig and Ward, (1997) who show that denture plastics of the same type may vary in water sorption because of the presence of additives. This result come in agreement with the finding of Peter and Ernst, (2004); Stafford, (1980) when examined the physical properties of the different brands of heat cured denture base resins that had different additives. The resins exhibit significant differences in residual MMA monomer, water up take.

This result disagree with Mohammad, (2010) who show that the addition of chemical materials to the Major acrylic resin produce a statistically significant increase in its water sorption and solubility.

5.7. Tensile Strength Test

The tensile strength result of heat cured acrylic resin(HCAR) groups and Duncan's multiple range tests of it presented in figure(4.10), showed that the control sample (HCAR) only without additive has the best tensile strength followed by (HCAR) mixed with 2% Hydroxy appetite, and 5% (HA) respectively.

This study agree with Deb *et al*., (1995) who show that after immersion of the sample in the water, the tensile strength was found to decreased due to the absorption of water which acts as plasticizer. But disagree with

Vallitu , (1998) who show that the addition of loose glass fiber to PMMA increases the tensile strength of the reinforced denture.

5.8. Dimensional Accuracy Test

The Paired T-test between means shown in table (4.6) showed no significant difference between all tested groups.

The resins were all found to shrink during the curing process. However, on immersion in water, the resins were found to expand. He reported greater shrinkage during processing than during storage in water up to 90 days (Shippee, 1961; Anusavice, 2004; Noort, 2005).

This study agree with Komiyama and Kawara, (1998) stated that the stress produced by thermal contraction is relieved shortly after the denture has been removed from the mold and that stress, caused by polymerization contraction, will be relieved more gradually. This study disagree with (Craig and Ward, 1997; Ganzarolli *et al*., 2002; Kobayashi *et al*.,2004) They concluded that the thermal contraction stresses are of instantaneous mechanical nature, where as the stresses caused by polymerization are on a molecular level involving polymer chains the water immersion of the acrylic samples caused expansion of the acrylic resin which partially but not completely compensate for the processing shrinkage. whereas the stresses caused by polymerization are on a molecular level involving polymer chains. The stress caused by thermal contraction was relaxed by molecular reorientation of the polymer chains as state by Kobayashi *et al*., (2004).

5.9. Residual Monomer Test

The residual monomer test result shown in figure (4.12) that show there were no release of residual monomer in (HCAR) mixed with 2% and /or

5%(HA) , but there is residual monomer release up to fourth day in both (HCAR) relined with (HA) and control group.

The solubility is a property of acrylic resin, representing the not reacted substances releasing (residual monomer, plasticizers and initializers). It is of resins, since they should be insoluble in oral fluids. Residues releasing from a polymerized resin base can promote tissular reactions in users of prosthesis (Machado *et al.*, 2002). According to this point of view, the degree of conversion is one of the most important characteristic of resin, on account of the high residual monomer levels that could be un reacted. Its presence has an adverse effect on physical and mechanical properties (Yunus *et al.*, 1994). Acrylic resin also presents water sorption that is directly related to the polar properties of resin molecules, the physical process of water diffusion through intermolecular space (Takahashi *et al.*, 1998), and the amount of residual monomer in the polymerized mass (Wong *et al.*, 1999). Thus, Polymerization degree is directly related to resin's ability of absorbing water (Meloto *et al.*, 2006). Polymerization takes place as the free radicals open the double bonds of the methyl methacrylate, creating a chain reaction where the monomer attaches to polymer free radicals.

CHAPTER SIX

CONCLUSIONS AND SUGGESTIONS

6.1 Conclusions:

The present study comes up with the following conclusions:

1. Adding Hydroxyapatite to heat cured acrylic resin increase transverse strength and surface hardness, but decrease water sorption and solubility and tensile strength.
2. Adding Hydroxyapatite to heat cured acrylic resin show there is no change of dimension and decrease in residual monomer release.
3. In FTIR test showed that the addition of 2% and /or 5% hydroxy apatite to heat cured acrylic resin denture base material resulting a homogenous mixture, without chemical reaction.
4. Using Hydroxyapatite as additve for denture lining show biocompatibility of the material and enhance new bone formation**.**

6.2 Suggestions:

1. Comparison between industrial Hydroxyapatite and powder Hydroxyapatite of egg shell.
2. Effect of addion of Hydroxyapatite on color of relined heat cured acrylic denture base**.**
3. Evaluation of other mechanical and physical properties as impact strength.
4. Attachment of artificial teeth with different material with (HA) containing denture base .
5. Testing higher (HA) concentration.

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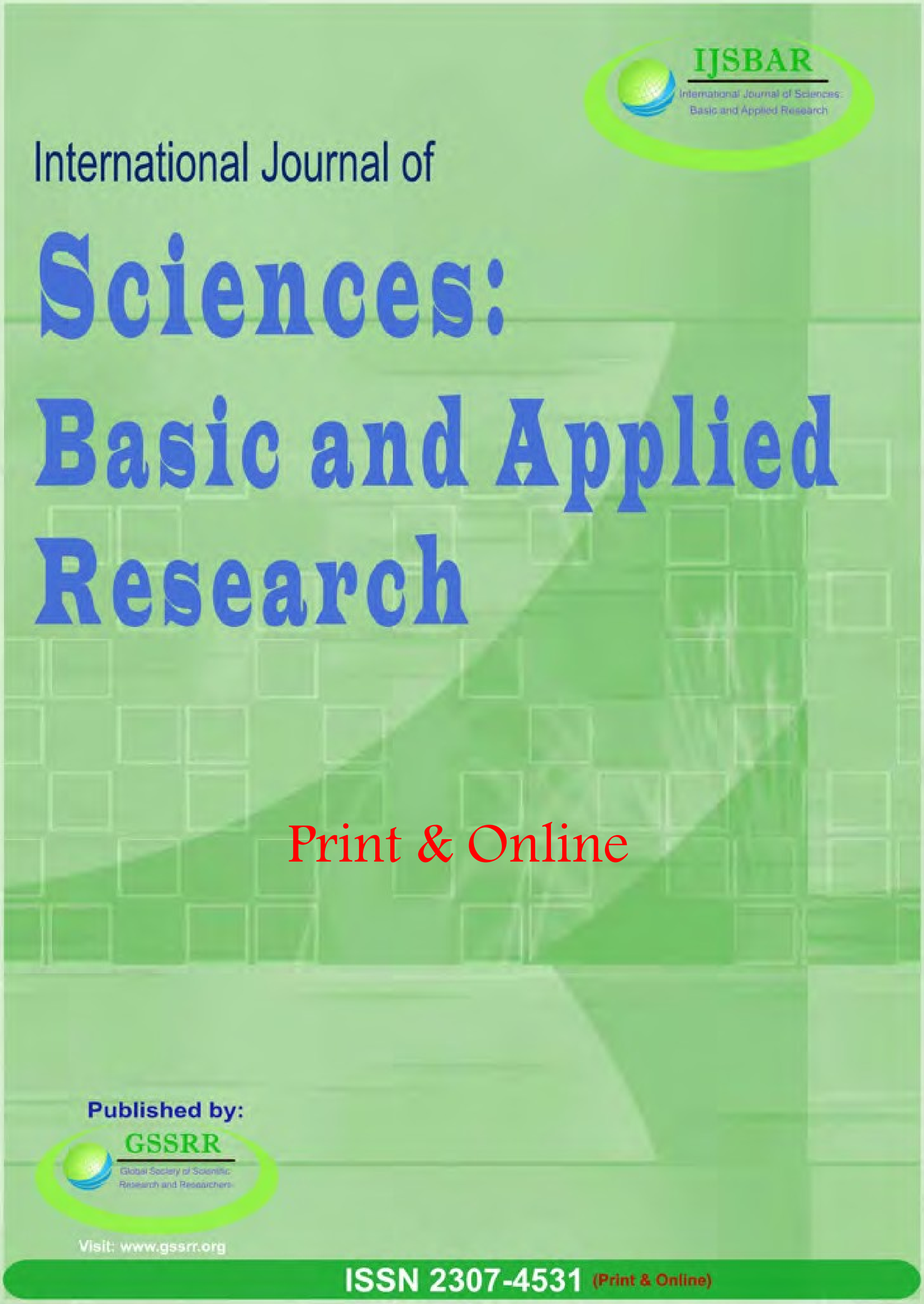

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