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Preparation of Local Gingival Shade Guide by Using Natural Pigments

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University of Mosul

College of Dentistry

Preparation of Local Gingival Shade Guide by Using Natural Pigments

A Thesis Submitted by
Nashwah Subhi Azeez Al-Ibrahim

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

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ABSTRACT

Introduction: There is a need for the development of color additives by manufacturers, and dental clinics to tint acrylic powder resins to match the observed gingival colors. Some natural pigments (Amaranth, Raspberry, Titanium dioxide, Vanilla, and Curcumin) are low in price, and available in our country. The influence of these products on matching artificial heat cured acrylic gingival shades has not been tested before.

Aims: The aims are to use natural pigments (Amaranth, Raspberry, Titanium dioxide, Vanilla, and Curcumin) in different concentrations instead of the Vertex™ synthetic acrylic stains, with heat cured acrylic resin denture base material. Then matches the natural pigments colors with natural human gingival colors, and correlate with Vertex™ gingival shade guide and Vertex™ synthetic stains colors, also detect the influence of these products on some properties of heat cured acrylic resin denture base.

Materials and Methods: A total of (1022) samples were prepared of translucent, and pink heat cured acrylic resin Vertex™ material that were divided into 3 groups; The 1st group (control translucent, control pink acrylic) without additives. The 2nd group included samples prepared from translucent and pink acrylic with natural pigment additives (Amaranth, Raspberry, Titanium dioxide, Vanilla, and Curcumin). The 3rd group included samples prepared from translucent and pink acrylic with Vertex™ synthetic acrylic stains.

The effect of natural additives was compared with synthetic additives by measurement of color property, indentation hardness test, volumetric

changes test, water sorption and solubility, residual monomer concentration, FTIR test and microscopic texture examination.

The colors of all prepared samples were correlated with the Vertex™ gingival shade guide, and gingival color of 24 healthy young participants selected from the College of Dentistry – University of Mosul, by using Vita Easyshade device, then calculate (ΔE) between them (in between). Statistical analysis was managed by SPSS software, and a special designed program by MATLAB (2010).

Results: Results of color property test showed that color of some samples with natural pigments matched the color of patient's gingiva, samples with synthetic stains, and gingival shade guide that (ΔE) between matched values is ≤ 6.8 which accepted in vivo color change.

Results showed significant differences at ($P<0.05$) between control group (samples without additives) and groups with both natural and synthetic™ coloring agent additives in an indentation hardness test, water sorption and solubility, residual monomer concentration. While volumetric changes test showed no significance differences at ($P<0.05$).

Conclusions: The results approved that the use of the natural pigments (Vanilla 10%, Amaranth 0.1% and mixture of Amaranth with Vanilla in different concentrations) instead of the Vertex™ synthetic acrylic stains No. (210, 220, 230, 240) in different concentrations are clinically acceptable compared in relation to patients attached gingival color and Vertex™ gingival shade guide. All the results of studied mechanical, chemical and physical properties of all tested groups were within the range of acceptance according to ADA specification No.12, (2002).

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List of Abbreviations

Abbreviation	Name
%	Percentage
°C	Degree centigrade
3D	Three dimensions
a	degree of redness and greenness
Abs	Absorbance
ADA	American Dental Association
ANOVA	Analysis Of Variance
b	Degree of yellowness and blueness
C	Chroma
C=C	Carbon-Carbon double bond
C-C	Carbon-Carbon single bond
C=O	Carbonyl group
CIE	Commission Internationale de L' Eclairage
conc.	Concentration
df	Degree of freedom
FAO	Food and Agricultural Organization
FDA	Food and Drug Administration
FTIR	Fourier transform infrared spectra
g/day	Gram per day
gm	Gram
H	Hue
IR	Infrared
ISO	International Standard Organization
KBr	Potassium bromide

Kg	Kilogram
L	Lightness
mg	Milligram
ml	Milliliter
mm	Millimeter
MMA	Methylmethacrylate
m	Mass
µg	Micro gram
µpa	Mega Pascal
N	Number of sample
nm	Nanometer
No.	Number
P	Probability
PMMA	Poly methylmethacrylate
SD	Standard Deviation
Sec	Second
TiO_2	Titanium dioxide
™	Trade mark
UV	Ultraviolet
Vs	Versus
V	Volume
WHO	World Health Organization
wt	Weight
wt/wt	Weight per weight
ΔE	Color difference
λ	length Wave

CHAPTER ONE

INTRODUCTION

Poly methylmethacrylate (PMMA) is currently the material of choice for denture base fabrication. During fabrication of a denture, the physical and mechanical properties are influenced by the choice of materials. Dentists and manufacturers of denture base materials have long been searching for ideal materials and designs for dentures (Mohamed *et al.*, 2008).

In order to obtain a natural looking restoration, there are two crucial steps in everyday dental practice; the selection of color through shade guide which will harmoniously integrate itself with surrounding biological tissue and consequently the correct reproduction of the color in the prosthesis (Corciolani, 2009). Acrylic resins possess the advantages of good pigment wettability, high gloss and so on (Xu *et al.*, 2009).

It is not only important to have good dental esthetics, but also good gingival esthetics. Esthetic considerations in prosthetic restorations, such as fixed implant-supported, and removable prostheses, have focused primarily on the anterior region. An attractive or pleasing smile involves the harmonious interaction of three primary components; the lip position, teeth, and associated gingival architecture (Tjan *et al.*, 1984).

The modest interest in the topic of gingival color is most likely a consequence of the difficulties of finding a suitable system for soft tissue color measurement and matching (Bayindir *et al.*, 2009). The color of the healthy gingiva is assumed to vary from pale pink to bluish purple, coral pink, light brown and dark brown, between these limits of normalcy are a large number of colors, the exact line being a matter of individual variation (Oluwole and

Elizabeth, 2010). The potential to achieve a good visual shade match of gingival tissue is different for various racial groups (Bayindir *et al.*, 2009).

Oluwole and El izabeth (2010) stated that "There is a need for the development of chemical additives by manufacturers and dental clinics to tint acrylic powder resins to match the observed gingival colors ". Many attempts have been made to give a natural appearance to the denture base, which includes application of various coloring agents. The methods used to assess gingival color may be subjective, they may still be useful guides for the development of more natural appearing denture resins.

AIMS OF THE STUDY

The aims of this study were:

1. To prepare local artificial gingival shade guide by using natural pigments instead of that available from synthetic stains. This by finding the preferable concentrations of some natural pigments (Raspberry, Amarath, Titanium dioxide, Vanilla and Curcumin) with heat cured acrylic denture base material then compared with Vertex™ synthetic stains and Vertex™ artificial gingival shade guide.
2. To identify the color property of natural additive pigments on heat cured acrylic resin denture base.
3. To study the effects of these incorporations on some physical, chemical and mechanical properties (color property, hardness, volumetric change, water sorption and solubility, residual monomer, FTIR and microscopic examination) on the heat cured acrylic denture base material.

Chapter Two
Literature Review

2.1 Definitions

- **Color:** A visual response to light consisting of the three dimensions of hue, value, and saturation (Academy of Prosthodontics, 2005).
- **Hue:** The attribute of color by means of which a color is perceived to be red, yellow, green, blue, purple, etc (Academy of Prosthodontics, 2005).
- **Value:** The dimension of a color that denotes relative blackness or whiteness (grayness, brightness) (Academy of Prosthodontics, 2005).
- **Chroma:** The purity of a color or it's the saturation of the hue (Academy of Prosthodontics, 2005).
- **Color difference:** Magnitude and character of the difference between two colors under specified conditions; referred to as delta ΔE (Academy of Prosthodontics, 2005).
- **Natural coloring agents:** Chemical substances extracted, isolated or otherwise derived – with intermediate from vegetable, mineral, animal or any other source, which when added to a product is capable (alone or through reaction with other substance) of imparting a distinguishing color thereto (National board for drug selection, 2011).
- **Synthetic coloring agents:** Chemical substances produced by synthesis or any other artifice and which when added to a product is capable (alone or through reaction with other substance) of imparting a distinguishing color thereto (National board for drug selection, 2011).

2.2 Acrylic Resin (Poly methylmethacrylate)

Acrylic based resins are intensively used in dentistry practice as restorative, liners or in fabrication of removable partial and complete denture bases materials (Ali and McLean, 1999).

This substance is made by polymerization of methacrylate related monomers, it can be classified as chemical, heat or light polymerizing depending on the factor that initiates the polymerization reaction (Bettencourt *et al.*, 2010; Morozova, 2010). The heat cured denture base resins are extensively used for their good properties, while light polymerizing recommended especially for patients with hypersensitivities to poly methylmethacrylate (PMMA) (Gohlke *et al.*, 2011; Wehrbe *et al.*, 2012).

The advantages of poly methylmethacrylate (PMMA) include excellent esthetic properties, adequate strength, low water sorption, low solubility, facility of repair and construction by a simple molding and processing technique (Parvizi *et al*., 2004).

Other materials than acrylic with superior properties have been on the market for a limited time, acrylic still remains the most popular choice, the reason for this continued popularity is the simple processing equipment required and the relatively low cost of the fabrication process (Cunningham, 2000). Increasing concern of these material arises regarding the safe clinical application of these materials due to their biodegradation under the oral environment (Bettencourt *et al.*, 2010).

Since the introduction of PMMA as a denture base material, the ultimate goal of prosthodontists has been to improve denture naturalism, involving basically the reproduction of the contour, texture and color of the patient's gingival tissue in the denture base, to date up to 95% dental

prostheses are composed of PMMA, due to its advantages, including its optical properties, biocompatibility, and aesthetics (Tanoˇ glu and Ergˇ un, 2007; Gurbuz *et al.*, 2010).

One shade of pink for partial dentures is not acceptable for all patients, especially when the acrylic flange is adjacent to gingiva in the esthetic zone. The color of denture base acrylic resin is not as critical in complete dentures. Fortunately, individual tinting of denture base resins has been described using stain kits (Haeberle and Khan, 1997).

2.2.1 Composition of Acrylic Denture Base Materials

These are supplied in powder-liquid form, which upon mixing and subsequent heating form a rigid solid. The powder contains poly methylmethacrylate beads along with initiator (benzoyl peroxide), plasticizer (dibutyl phthalate), pigments, opacifiers and synthetic fiber (nylon/acrylic), the liquid contains methylmethacrylate monomer with inhibitor (hydroquinone), cross-linking agent (glycol dimethacrylate) (Noort, 2002).

Currently, almost all denture materials are radiolucent and concerns exist about the difficulty of removing fragments of fractured dentures aspirated during accidents. Addition of Bismuth (10-15%) or uranyl salts provides adequate radiodensity, but at the cost of increased transverse deflection and water sorption (Tandon, 2010).

2.2.2 Additives to Acrylic Resin Denture Base Materials

Strength, accuracy, porosity, residual monomer, color and other properties of heat cured denture base resins are field for ongoing researches, leading to various modifications to improve its strength, antimicrobial, color and other properties either by different curing cycle and curing methods

(Pronych *et al.*, 2003; Lung and Darvell, 2005), or by using additives materials such as glass flake polyethylene fiber, polybutene a reactive plasticizer, coloring agents (Siphi *et al.*, 2006; Hautamaki *et al.*, 2010; Uematsu *et al.*, 2010).

Effective fiber reinforcement depends on many variables including the material used, the percentage of fibers in the matrix and their modulus and distribution, fiber length, fiber orientation and fiber form. The addition of fibers to acrylic resin has the potential to improve the mechanical properties of the material, that fibers substantially increased the impact strength of PMMA resin (Ladha and Shah, 2011; Mowade *et al.*, 2012; So *et al.*, 2012).

Over years, various types of fibers are used such as carbon fibers (Bowman and Manley, 1984), aramid fibers (John *et al.*, 2001), metal wires (Kanie *et al.*, 2003), glass fibers (Kim and Watts, 2004; Basant and Reddy, 2011), silver nanoparticles (Vodnik *et al.*, 2009), and polyethylene (Mikhailova *et al.*, 2012; Mowade *et al.*, 2012; Nuinu *et al.*, 2012).

Bashi and Al–Nema (2009) concluded that all forms of fiber reinforcement and metal acted to reduce the hardness of acrylic denture base resin.

Improved tensile and flexural strengths were observed in PMMA with 10% and 20% silver fillers and 10% aluminum fillers (Matinlinna *et al.*, 2009; yaday, 2012), and with natural oils (Ramanen *et al.*, 2012).

Hatim *et al.* (2010) added pure natural oils to the acrylic resin as oil of nigella sativa and thyme to give an acceptable antimicrobial effect and showed no effect on the color after curing of the acrylic resin denture base.

Hatim *et al.* (2004) studied the color property and concluded that there are no significant differences in optical density (color property) for acrylic resin cured by microwave and water bath techniques.

Still PMMA material is not ideal in every respect and it is the combination of virtues rather than one single desirable property that accounts for its popularity and usage. Despite satisfying esthetic demands, it is far from ideal in fulfilling the mechanical requirements of prosthesis (Mowade *et al.*, 2012).

2.2.3 Coloring Agents

Coloring additives are obtained as natural material and synthetic products (Craig *et al.*, 1985).

Esthetic effects are sometimes produced in a restorative material by the incorporation of colored pigments, this means pigments in non metallic materials such as composites, denture acrylics, silicone maxillofacial materials and dental ceramics, the color observed when pigments are mixed results from the selective absorption by the pigments and the reflection of certain colors (Powers and Sakaguchi, 2006; Pattanaik and Pattanaik, 2011).

Several pigments, such as carbon black, zinc oxide, titanium dioxide, and cadmium red, added to acrylic resins in order to match their color to that of patient's gingiva (Zimmerman, 1982).

There is an increasing consumer concern about health and safety issues related to the use of synthetic colorants. Their use is restricted in several countries. In addition, national authorities such as The Food and Drug Administration (FDA) in USA have restricted the use of several synthetic colorants, suspected of being responsible for increasing the probabilities of the induction of allergenic reactions. Other colorants are under study and are provisionally allowed, increasing the world tendency to use natural additives. Blue colorant is especially important because of its low presence in nature and

the high demand in confectionary and drinks industry (Porras *et al.*, 2010; Gultekin and Doguc, 2012).

2.2.3.1 Natural Coloring Agents

The obtainment of coloring matter based on natural products is of considerable importance since the United States have banned the use of synthetic coloring in foods (Scoles *et al.*, 2000).

The reason for accelerating demand of the natural food colors in international market is the growing awareness of environmental hazards of synthetic colors and harmful impact of chemicals used for manufacturing them (Divya *et al.*, 2011; Siva *et al.*, 2011). European countries have not only put total ban on manufacturing of synthetic dye based colors and the products containing such colors, but also banned the imports of products from the countries using such colors. There are many important permitted natural colorants used in food, nutritious and pharmaceutical preparations among others such as (Curcumin, Amaranth, Red cherry, Saffron, Raspberry, etc) (National board for drug selection, 2011).

2.2.3.2 Synthetic Coloring Agents

Synthetic color additives requiring certification and the natural color additives exempt from certification, despite the importance of food colorants, there is an ever growing concern about the adverse effects of synthetic food colorants on human health (Van *et al.*,1989).

There has been a sharp increase in the use of synthetic colorants during the past few years and this use of synthetic colorants particularly in foods (Ganong, 1991).

Synthetic food colors is mainly benzene or xanthene structure of compounds made of benzene, toluene, naphthalene and other chemical products, which have some toxicity (Guo-qing *et al.,* 2009).

The synthetic colorants can be toxic if consumed in large amounts, thus each synthetic food colorant has been evaluated by the Food and Agricultural Organization (FAO) and World Health Organization (WHO), and there is an increasing need to monitor the levels of such dyes in various products (Nia *et al.,* 2009).

The vulnerable population should not be exposed unnecessary to excessive amounts of synthetic colors and the health risk they pose (Dixit *et al.,* 2011).

2.3 Some Properties of Heat Cured Acrylic Resin Denture Base Material

Restorative dental materials are developed by the producer and selected by the dentist on the basis of characteristics physical, chemical and mechanical properties of the materials, no single property can be used as a measure of quality of materials, often several combined properties, determined from standardized laboratory and clinical tests are employed to give a measure of quality (Craige and Powers, 2002).

The denture base resin is subjected to various stresses during function. During fabrication of a denture, the physical and mechanical properties influence by the choice of materials (Mohamed *et al.,* 2008).

2.3.1 Color Property

Matching the pink soft tissues of the mouth, various blends of red and white are necessary, with occasional need for blue, brown, and black

in small quantities, the color and translucency of human tissue show a wide variation from patient to patient (Sgar *et al.*, 1985).

Achieving a close shade match of an artificial restoration with the natural tissue is complex process and the accurate shade matching of tissue color is one of the most challenging aspects of dentist and clinician that shade selection errors can result (Ishikawa-Nagi *et al.*, 1994). An understanding of color, light and related characteristics of resin are required, as well as the ability to clearly communicate instructions with laboratory technicians (Corciolani, 2009). Then Dental color science developed in a manner that minimized the errors in visual color selection (Miller, 1993 and 1994).

The commission International del'Eclairage (CIE, 1931) released the standards for color matching, establishing some scientific parameters for color evaluation, but the absence of valid scientific instruments for color measurement allowed no significant improvements. Sproull (1973a. and 1974) published a serious of articles which the three dimensional nature of color, and its relationship with dental shade was studied and a series of theoretical and practical indications were given in order to improve color matching in dentistry. This was represented for a long time the "state of the art" for color matching, the science of color was greatly improved, the desire was to use science to express color and differences in color numerically. Since the Munsell system had an irregular space distribution, it was not possible to correlate two colors and to calculate the differences between them, in 1976 and 1978 the CIE developed a new system called CIE L*a*b* (Baltzer and Kaufmann, 2004).

Unfortunately, dental practice did not benefit immediately from this knowledge to improve color matching; visual assessment still considered the bench mark approach (Corciolani, 2009)

Color assessment of human gingival and mucosal tissues is an essential first step to develop an intraoral soft tissue shade guide. Necessary color measurements can be performed using visual, spectrophotometric, or photographic techniques. Knowledge of the distribution of gingival and mucosal shades is important to achieve individual customization of denture base color (Schnitzer *et al*, 2004).

When the colors are combined with proper translucency, to match the pink soft tissues of the mouth, various blends of red and white are necessary, with occasional need for blue, brown and black in small quantities, the color and translucency of human tissue shows a wide variation from patient to patient and from one area of the mouth to another (Powers and Sakaguchi, 2006).

2.3.1.1 Systems for Color Measurement

The Munsell system is the oldest color order system. Its use has been documented in dentistry (Sproull, 1973a; Sproull, 1973b; Matthews, 1978).

In 1976 other system emerged, recommended by the Commission International de l'Enclairage (CIE) and named CIE L*a*b*, this has been widely used for non-self-luminous objects such as textiles, paints and plastic objects (Baltzer and Kaufmann, 2004). Between 1976 and 1978 when the CIE L*a*b* developed, for the first time it was possible to classify and correlate color numerically, and to calculation the difference between two colors using formula that gives one number (ΔE) as value for color differences (Clarke, 1983).

(CIE, 1978) Color difference (ΔE) as value for color differences became critical in color science as well as in the color industry ranging from textiles to dentistry that calculated as following (Craige and Powers, 2002):

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad \ldots\ldots\ldots \quad (2\text{-}1)$$

$$\Delta E = [(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2]^{1/2} \quad \ldots\ldots\ldots \quad (2\text{-}2)$$

Several studies (Seghi *et al*., 1989; Vichi *et al*., 2004) provide information regarding clinical color matching tolerance. Johnston *et al*. (1989) determined clinically acceptable shade matching in oral conditions for (ΔE) up to 3.7.Vichi *et al*. (2004) reported value of (ΔE) < 1 as not appreciable by human eye.

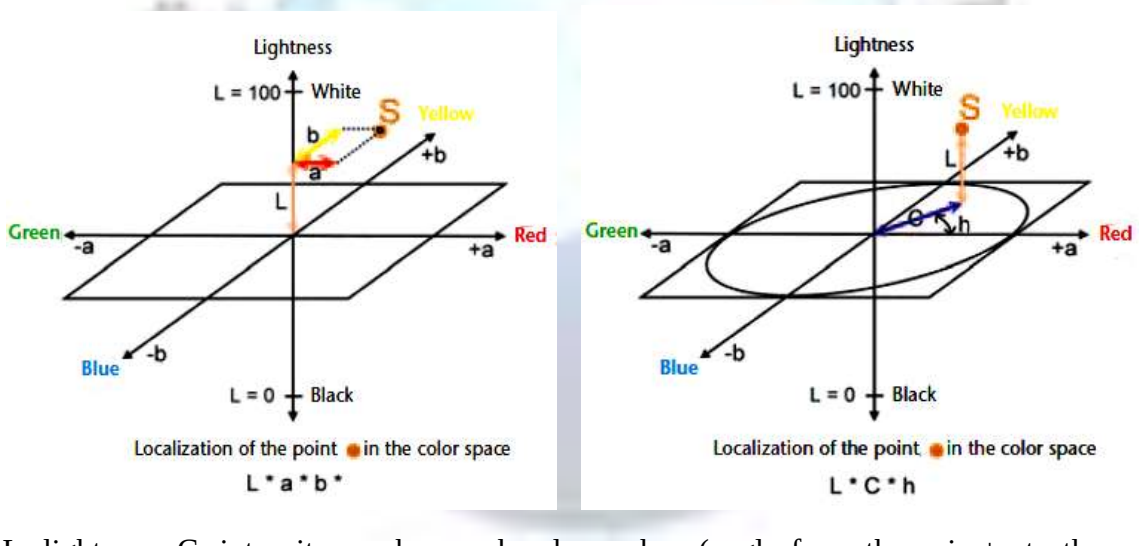
In the late 1990s, the l*a*b* system was adopted by dentistry and one of the first clinical results was the development of the Vita 3D Master shade guide (Corciolani, 2009).

CIE L*a*b* approach is considered an international model for measuring color. The model CIE L*a*b* represents a tridimensional color space with three axes: L, a, and b. The letter L* represents the measure of luminosity or clarity of an object, and it is quantified according to a scale on which the perfect black has a value of L* equal to zero, whereas the total white has a value of L* equal to 100. There are also two chromatic components, which represent variations in hue and chroma. The a* axis is measured from red (a* positive) to green (a* negative), varying, respectively. The b* axis is measured from yellow (b* positive) to blue (b* negative). The coordinates a* and b* approach zero for neutral colors (white, gray) and increase in magnitude for more saturated and intense colors (Ishikawa-Nagai *et al.*, 2004; Luo *et al.*, 2007).

Another illustration of the CIE L*a*b system can be easily achieved using the so-called L*C*h parameters. With this method, the distribution of the colors of the L*a*b color space remains unchanged; only the location of the color in the color space is calculated in a different way. In the L*a*b

system, (a) color location is defined by the distances on the coordinates L, a and b. In the L*C*h system, (a) color location is defined by the distance on the coordinate L (lightness, value, height of the color location in relation to the L-coordinate), with the degree C (color intensity, chroma, distance from the L-coordinate to the color point) and the angle h (color, hue, angle from the axis +a to the color location) as in Figure (2.1). Conversion between L*a*b and L*C*h is done according to the following formula (Baltzer and Kaufmann, 2004):

$$L^*C^*h^* \mid L \text{ (Value) remains } L \mid C \text{ (Chroma)} = \sqrt{a^2 + b^2} \mid h \text{ (hue)} = \sin(h) = b/\sqrt{a^2 + b^2}$$



L: lightness, C: intensity or chroma, h:color or hue (angle from the axis +a to the color location), a: degree of redness and greenness, b: degree of yellowness and blueness.

Figure (2.1): CIE L*a*b system achieved using L*C*h* parameters (Baltzer and Kaufmann, 2004).

Scientific study of dental color has been directed towards minimizing errors in visual color selections, primarily through the use two instruments; colorimeter and spectrophotometers (hunter and Harold, 1987).

Corciolani (2009) explained that the colorimeter is a relatively simple and low cost instrument designed to measured color on the basis of three axis or stimuli by the way of filter that simulates the human eye, while the spectrophotometer is a more sophisticated instrument, designed to measure an observed object by reflection or transmission, the results of which are the entire spectral curve limiting color measurements to a visible wavelengths range (usually 350-800 nm).

2.3.1.2 Spectrophotometric Measurements

The spectrophotometer is a sophisticated instrument, built to measure by reflection or transmission of an observed object, given the entire spectral curve and is limited for color measurement to the visible wavelengths range (usually 350-800 nm), significant advantages with spectrophotometric measurements include the ability to analyze the principal components of a series of spectra and the ability to convert spectrophotometric measures to various color measures (Corciolani, 2009).

Clinical dental spectrophotometers have been developed and marketed, such instruments are based on CIE lab color system, although they provide measurement in any dental shade system, their technology allow to convert perception in numbers, thus making color selection and color communication in dentistry easier and more reliable (Paul *et al*., 2002; Baltzer *et al*., 2005; Ishikawa-nagai *et al* ., 2005; Corciolani *et al*., 2006).

Shade matching by using spectrophotometer meets all the requirements for successful choice of shade in accordance to the physiology of color vision

and the science of color. That method for shade determination is recommended in everyday practice and surely characterizes the present and the future of restorative dentistry (Andjelković1 *et al.*, 2010).

Vita EasyShade device is one that used for spectrophotometer measurement, this instrument provides data obtained over the range of visible wavelengths (about 400 to 700 nm), captures the tristimuli L*C*h* and subsequently calculates the values of L* , a* & b* (Caneppele and Torres, 2011). This instrument allow an improved understanding of color perception and its correction with clinical aspects as well as measuring difficult parameters such as translucency, hue, and value (Corciolani, 2009).

Vita Easyshade is one of the latest spectrophotometers available for clinical use that the instruments software is programmed to give CIE lab and LCh color values (Corciolani, 2009). It is an electronic shade selection device that uses a light source and spectrophotometer to determine a shade, it is composed by a base unit and a hand piece. The color evaluation was done free hand simulating the clinical use (www.vident.com).

2.3.2 Indentation Hardness

The hardness test measures the resistance of a material to an indenter or cutting tool, it provides an indication of the resistance of the material to scratching or abrasion, the test involves the use of an indenter, which can be in the shape of a ball (Brinell), a pyramid (Vickers or knoop) or a cone (Rockwell) which of course must be harder than the material being tested, the indenter is pushed into the surface of the material for a given period of time, leaving behind an impression of the indenter, the size of the impression will depend upon the hardness of the tested material (Noort, 2002).

The hardness of denture base materials may undergo changes due to continued polymerization reaction. The degree of conversion of heat cured acrylic resin may be evaluated indirectly by measuring the surface hardness (Azevedo *et al.*, 2005).

2.3.3 Volumetric Change

Acrylic resin is the most commonly used material in dental construction, it is subject to polymerization shrinkage and distortion (Consani *et al.*, 2002). This resin shows a volumetric shrinkage of approximately 8% (Geerts and Jooste, 1993).

Dimensional accuracy is an important requirement of many dental materials, the success of many restoration procedures depends on dimensional changes, that may be due to water absorption by, or loss of constituents from the material (McCabe and Walls, 2008).

2.3.4 Water Sorption and Solubility

Water sorption and solubility are important properties of acrylic resin, denture base acrylic resin have two solubility. This solubility results from the leaching out of unreacted monomer and water soluble additives into oral fluids, the solubility of denture base can cause oral soft tissue reactions. In addition, water absorbed into this material acts as a plasticizer and decreases the mechanical properties such as hardness, transverse strength, fatigue limit and also can affect on dimensional stability (Tuna *et al.*, 2008).

Water sorption presumably occurs among macromolecules, which are forced slightly apart, this separation causes molecular mobility. Inherent stress created during heat curing of the acrylic resin can be relieved, with resulting intermolecular relaxation and possible changes in the shape of the denture, these properties are favorable for resin used in the elaboration of dentures because, after absorbing water, they provide more retention to the denture base in contact with the edentulous ridge (Barbosa *et al.*, 2001).

Poly methylmethacrylate is soluble in most solvents (e.g chloroform) as it is only lightly cross linked, it is virtually insoluble in most of the fluids that it may come into contact within the mouth, however some weight loss will occur due to leaching of the monomer in particular, and possibly some of the pigments and dyes (Noort, 2002).

Solubility of materials in the mouth and the sorption (adsorption plus absorption) of oral fluids by the material are important criteria in their selection frequently, laboratory studies have evaluated materials in distilled water (Craig *et al.*, 2004).

Water is absorbed by acrylic resin stays in gaps among inters polymeric chains that form acrylic resin structure. The magnitude of these inter polymeric gaps determines the amount of water to be absorbed, it affects the dimensional stability, subjecting the material to internal stresses and possible crack formation (Tuna *et al.*, 2008; Garcia *et al.*, 2010). Better polymerization of acrylic resin increases the cross linking and reduces water sorption values (MELOTO *et al.*, 2006).

ISO 1567:2001 standard (Dentistry: Denture base polymers) recommends water sorption lower than 32 $\mu g/mm^3$ within the established limit.

Simple method of assessing the water sorption and solubility of a polymers is to monitor the weight change of a sample when immersed in water, the detailed analysis of the amount of water sorption by polymeric materials is complicated by the concurrent loss of water–soluble components such as residual monomers or plasticizer, as these two processes take place simultaneously, although at different rates. It is important in the characterization of these factors that the two processes are separated (Noort, 2002).

Water sorption is usually measured gravimetrically in μg /mm^3 after seven days in water, usually warpage and dimensional change are associated with high percentage of water sorption (Sherwood, 2010).

In a denture base material, water absorbed acts as a plasticizer and affects the dimensional stability, subjecting the material to internal stresses and possible crack formation (Tuna et al., 2008; Garcia *et al.*, 2010).

2.3.5 Residual Monomer

Heat polymerized acrylic resin contain less residual monomer content than in autopolymerized acrylic resin and that reason is the low degree of conversion achieved by the use of a chemical activator as opposed to that generated by heat activation (Vallittu, 1996; Kabiri *et al.*, 2011; Lei *et al.*, 2011) .

Acrylic base resins are widely used in orthopedics and dental surgery, it is generally accepted, due to the incomplete conversion of methyl methacrylate (MMA) monomer to the polymer form during polymerization of the resin, some MMA monomers remain in the hardened material. MMA

monomer has been reported to cause abnormalities or lesions in several organs of animals (Abdi *et al.*, 2005).

The various storage conditions influenced the rate of leaching of MMA, for the acrylic samples stored in water and artificial saliva the elusion decreased with time for approximately 4-5 days. A time associated effect was found as the residues extracted many materials and under most storage conditions became noticeably smaller with time, the main variations between resins occurred according to the storage conditions. Some differences are observed between brands with respect to the amounts of the additives present that were leached, probably due to the differing chemical formulations of the products (Sofou *et al.*, 2005).

The low concentration of residual monomer is due to that the release of residual monomer is temperature dependent process, that increasing the temperature enhances the diffusion (Sadoon *et al.*, 2007).

During clinical use, the denture base materials are immersed in saliva and when not in use may be soaked in water. When immersed in such solutions, plasticizers and other soluble components may leach out over extended periods, while water or saliva is absorbed. The loss of plasticizer may cause brittleness and increased hardness values (Mohamed *et al.*, 2008).

2.3.6 FTIR Test

While a number of studies have evaluated the FTIR of dental composites, little information is available regarding denture base acrylic resins (Urban *et al*., 2007 a).

The degree of conversion is expressed as a percentage of unreacted C=C bonds (Cekic - Nagas *et al*., 2008).

Spectroscopic study of organic compounds is an investigation of the types of waves that can be absorbed by the molecules of the compound, by exposing a sample of the material to a spectrum of known wavelengths, and investigating which frequencies it absorbs, a great deal about the structure of the molecules of the material can be learned (Parikh, 1974).

Infrared spectroscopy may be used to identify the composition of polymers, to monitor polymerization processes, to characterize polymer structure, to examine polymer surfaces and to investigate polymer degradation processes (Stuart, 2004).

The wavelength of the infrared region extends from approximately 750 nm (0.75 μm) to almost (830 μm), but in IR spectroscopy, usually only a small range (between 2.5 to about 15.4 μm) is employed. This region is often called the fundamental region (Parikh, 1974).

The infrared spectrum can be divided into three main regions; the far infrared (<400 cm^{-1}), the mid-infrared (4000–400 cm^{-1}) and the near-infrared (13 000–4000 cm^{-1}) (Stuart, 2004).

Abdul Razzak (2010) studied FTIR test for the acrylic samples prepared with natural additives and concluded that raising temperature and extended polymerization time of heat cured acrylic resin showed improved conversion, and lowering the monomer release.

2.4 Some Natural Color Additives for Acrylic Resin Denture Base Material:

2.4.1.Amaranth

The name Amaranth is derived from the Greek word Amarantos, or unfading, because of the ancient belief that the plant was immortal (Jonnalagadda *et al.*, 2004).

Grain Amaranth (*Amaranthus paniculatus*) is a pseudo cereal grow in clusters, rich in lysine, methionine, dietary fiber, calcium, iron and squalene and is used as a breakfast cereal (PUNITA and CHATURVEDI, 2000; Aphalo *et al.*, 2009; Das, 2012). It is a long cultivation history in Central and South America, which produces very small seeds with high nutritional benefits (Kalinova and Dadakova, 2009; Kong *et al.*, 2009; Ortega *et al.*, 2012).

The dye from Amaranth has been used for millennia to color everything from maize to the rouge painted cheeks of South African women during festival season (Jonnalagadda *et al.*, 2004). Then is also a synthetic dye that has been named" Amaranth" for its similarity in color to the natural Amaranth pigments known as betalains (Wikipedia, 2007).

The anthocyannin (reddish) pigments in Amaranth appear to have great potential as a source of natural, non–toxic red dyes, this pigment is used in food industry (Myers, 2002; Ghodake *et al.*, 2011).

The obtainment of coloring matter based on natural products is of considerable importance since the United States have banned the use of synthetic coloring in foods. Thus, the tinted Amaranth is of interest due to the fact that dyes for food which are not artificial are needed (Scoles *et al.*, 2000). It is likely to prove useful for applications in the food, plastics, cosmetics and other industries (Ruskin *et al.*, 1984), used in dental field as additive to the dental modeling wax (Alubaidi, 2008).

2.4.2.Raspberry

Scientific Name of Raspberry (*Rubus ideaus L.*) is a member of the Rosaceae family, grown as a perennial crop (Agar and Streif, 1996; Patamsytė *et al.*, 2010), a wild edible fruit, was analyzed for phenolic

contents, and antioxidant, antibacterial and antiproliferative activities (Saini *et al.*, 2012; Storozhok *et al.*, 2012).

Raspberries are among the most delicious and delicate small fruits are summer and fall bearing Raspberries (Strik, 2001). It is used as coloring and flavoring agent in medicines and foodstuffs (National board for drug selection, 2011)

The two main types of Raspberries are red and black. Yellow-fruited Raspberries result from a mutation of red Raspberries that prevents the formation of red color; they are grown exactly the same as red Raspberries. Purple Raspberries are a hybrid between black and red Raspberries. Red Raspberries, Rubus idaeus, are native to northern North America and Eurasia. Cultivated red Raspberries were introduced into the U.S. as long ago as 1771 (Finn and Strik, 2008).

2.4.3. Titanium Dioxide (TiO_2)

The other name of titanium dioxide is (white dye 6). It is a natural coloring agent and first approved in 1996 to be used as coloring agent for drugs, and in medical application in 1986. No adverse effect are known that used as food coloring additives (not to exceed 1% by wt) (National board for drug selection, 2011).

Titanium dioxide is Fine white powder, ninth most abundant element in the world, it is five times less abundant than iron, but 100 times more abundant than copper (Windholz, 1983). It has particle sizes that range from 0.1-4 μm (Boffetta *et al.*, 2001; Wren *et al.*, 2010).

TiO_2 as it is found in mineral sands that white in color. These sands are widespread throughout Western Australia, and they contain a range of minerals which are of value (FESA, 2009).

It is considered a low-cost, clean photocatalyst with chemical stability and non toxicity and has been used for a wide variety of environmental applications, including water treatment (Li *et al.*, 2008) and air purification (Sikong *et al*, 2010).

It is an extremely stable, unreactive powder, which interacts with light in a way that makes it one of the 'whitest' white materials available. It is ideal to use as a white pigment in both oil and water-based paints, as well as in paper, plastics, inks, and cosmetics. It is even allowed to be used this way in food and medicine (FESA, 2009).

In recent years, TiO_2 particles have been employed through a synthesis of PMMA that have been largely investigated for their activity as antimicrobial additives (Torres *et al.*, 2011).

TiO_2 particles modified by hyper-dispersant can be well dispersed in acrylic polymer (Wang *et al.*, 2007). Introduction of TiO_2 for preparing acrylic resins allows the production of polymer with both color and surface modifications (Torres *et al.*, 2011).

2.4.4.Vanilla

Natural Vanillin $C_8H_8O_3$ (benzaldehyde,4-hydroxy-3-methoxy) is a colonially propagated crop, it is one of the most common aromatic flavor chemicals, and is used in a broad range of flavors (Janarthanam and Seshadri, 2008). It occurs in the Vanilla bean at a level of 20g kg^{-1} dry weight and is

associated with many other compounds. Approximately, 12000 tons of Vanillin is consumed annually, of which only 20 tons is extracted from Vanilla beans (Krings and Berger, 1998; LUBINSKY *et al.*, 2008; Naidu *et al.*, 2012). The rest is produced synthetically, mostly from petrochemicals such as guaiacol and lignin (Clark, 1990).

Vanilla, which originated in Mexico, is a tropical orchid belonging to the family Orchidaceae (Childers *et al.*, 1959). The fully-grown mature fruits of Vanilla, also called beans or pods, develop characteristic aromatic properties by the process of curing. The cured beans are referred to as Vanilla (Rao and Ravishankar, 2000).

Natural Vanilla is a complex mixture of flavor components extracted from the cured beans of the Vanilla plant; V planifolia and V tahitensis, more than 170 volatile aromatic components have been identified (Rao and Ravishankar, 2000). It is one of the most popular fragrances in the food industry (Firmenich and Corporate, 2009).

Vanilla had the inhibitory effect against L. monocytogenes and Sal. typhimurium, leading to its further application in food products, such as fresh-cut fruits and vegetables, in order to replace the use of chemical preservatives (Krasaekoopt *et al.*, 2010).

2.4.5.Curcumin

The name Curcuma is derived from the Arabic word *kurkum*, which originally meant saffron, but is now used for turmeric. Turmeric is the yellow component of curry powder. It is the dried, ground rhizomes of Curcuma longa. Turmeric contains three pigments; the major one is called Curcumin and the two others are derivatives of turmeric, Curcumin is an important

permitted natural colorant used in food, nutritious and pharmaceutical preparations among others (Sowbhagya *et al.*, 1998). Structure of Curcumin (R1,R2 = OCH3) showed in Figure (2.2) (Mortensen, 2006).

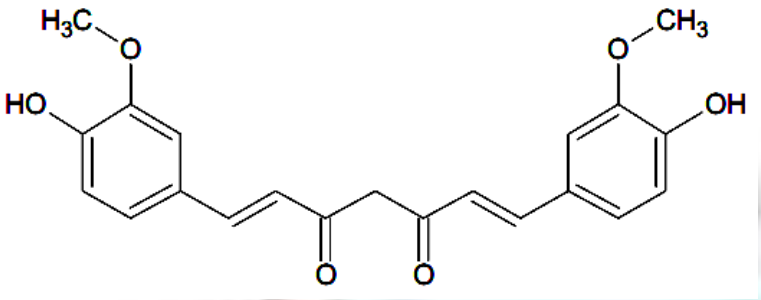


Figure (2.2): Chemical structure of Curcumin (Mortensen, 2006).

Curcumin chemical formula is $C_{21}H_{20}O_6$ (diferuloylmethane), it has been shown to have a wide spectrum of biological actions; these include its anti-inflammatory, antioxidant, anticarcinogenic, anticoagulant, antidiabetic, antibacterial, antifungual, antiprotozoal, antiviral, hypotensive and hypocholesteremic activities. The coloring principle is the main component of this plant and is responsible for the anti-inflammatory property (Chattopadhyay *et al.*, 2004). Human clinical trials also indicate that the Curcumin has no toxicity when administrated at dose of 10 g/day (Aggarwal *et al.*, 2003).

Curcumin has strong coloring power, safety and innocuity, comprehensive pharmacological function, but its low stability and water insoluble limit its application (Wang *et al.*, 2009).

CIE XYZ and CIE lab system color diagram in the cases of the microemulsion with Curcumin showed that trichromatic parameter values do

not change significantly from those in the case of dye solution, which shows a very good stability of their color (Cretur *et al.*, 2011).

2.5 Vertex^{TM} Acrylic Synthetic Color Additives for Acrylic Resin Denture Base Material

VertexTM synthetic acrylic stains give individual gum color characterization to denture base, Vertex™ stain kit contains (100g) each of the five colors (stain No. 210 (bleached pink), stain No.220 (pale pink), stain No.230 (pink), stain No.240 (purple), stain No.250 (brown) Shade) that are used to give an individual color characterization to full and partial dentures. This acrylic stains are suitable for use in combination with most denture base materials for the pressing technique and the pouring technique (www.vertex.dental.com). Colors are incorporated into, and polymerized with base material use with heat or cold cured acrylic (www.bracon.co.uk).

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CHAPTER THREE

MATERIALS & METHODS

3.1 Materials:

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

Table (3.1): Materials Used in this Study.

	Material	Manufacture	Certification	Expired date
1	Vertex™ regular Heat –cured resin powder and liquid (translucent and pink)	Vetrex™ – Dental bvJohan Van Oldenbamevertlaan, 62,3705 HJ Zeist the Netherlands	ISO 1567 Type 1 Class 1	2016–4
2	Natural pigments: Amaranth	India		-
	Raspberry	England		
	Titanium dioxide	England		
	Vanilla	China		
	Curcumin	India		
3	Vertex™ synthetic acrylic stains	Vetrex – Dental bv Johan Van Oldenbamevertlaan, 62,3705 HJ Zeist the Netherlands	YH472P03 ISO 1567	2014–7
4	Vertex™ separating medium	Vetrex – Dental bv Johan Van Oldenbamevertlaan, 62,3705 HJ Zeist the Netherlands	YG501C02	2012
5	Plastic foil (4mm, 3mm, 2.5mm)	Germany	Ch.No.4701A	-
6	Dental stone	Elite stone (Zehrmack) Italy	ISO 6873	-
7	Cotton.	Kardelen /Turkey	6027	-

8	Distilled water	Iraq		-

Figure (3.1) A: Materials Used in this Study, B: Natural pigments.
A; Raspberry, B; Curcumin, C; Vanilla, D;Ttitanium dioxide and E; Amaranth.

3.2 Equipments and Instruments Used in this Study Listed as Follow:

1. Brush (16 Amigo).
2. Cutter.
3. Dental metal flask (Ash, England).
4. Dental vibrator (BEGO, Germany).
5. Digital vernier (accuracy 0.001mm) (IOS-USA).
6. Electrical sensitive balance (sensitivity 0.001 g) (Mettler Toledo, Switzerland).
7. Glass slab.
8. Glass jar.
9. Hydrolyic press (BEGO, Germany).
10. Incubator (Memmert GmbH +CO KG, Germany).
11. Metal mesh No. 100μ (England).
12. Portable engine and hand piece (QD, DEROTORS, UK).

13. Reflecting light microscope (Lomo Micmed 2, Russia).
14. Rubber bowel.
15. Ruler.
16. Rockwell hardness tester (Brooks, Germany).
17. Spatula.
18. Spectrophotometer (Fourier transform infrared spectroscopy) (BRUKER TENSOR–27, Germany).
19. Ulta Violet – Visible Spectrophotometer (DUAL 8 AUTO CELL UVS-2800 LABOMED, INC-USA).
20. Thermostatically controlled curing unit (Derotor, Multicure Qyale Dental, England).
21. Vertex™ gingival shade guide (Netherlands).
22. Vita Easyshade device (Vita Zahnfabrik, Germany).
23. Wax knife.

3.3 Methods:

3.3.1 The Experimental Design of Main Study

Total numbers of samples prepared in this study were (1022) samples. All samples were prepared from Vertex™ heat cured denture base acrylic resin material; samples were divided into three main parts shown in Figure (3.2):

- Part one: Thirty eight samples of heat cured resin prepared from translucent and pink denture base resin material without additives.
- Part two: Three hundred and seventy six samples of heat cured resin prepared from translucent and pink denture base resin material with synthetic Vertex ^{TM} acrylic stain additives as shown in Figure (3.3).

- Part three: Six hundred and eight samples of heat cured resin prepared from translucent and pink denture base resin material with natural pigments (Amaranth, Raspberry, Vanilla, Titanium dioxide, Curcumin) as additives (Figure 3.4).

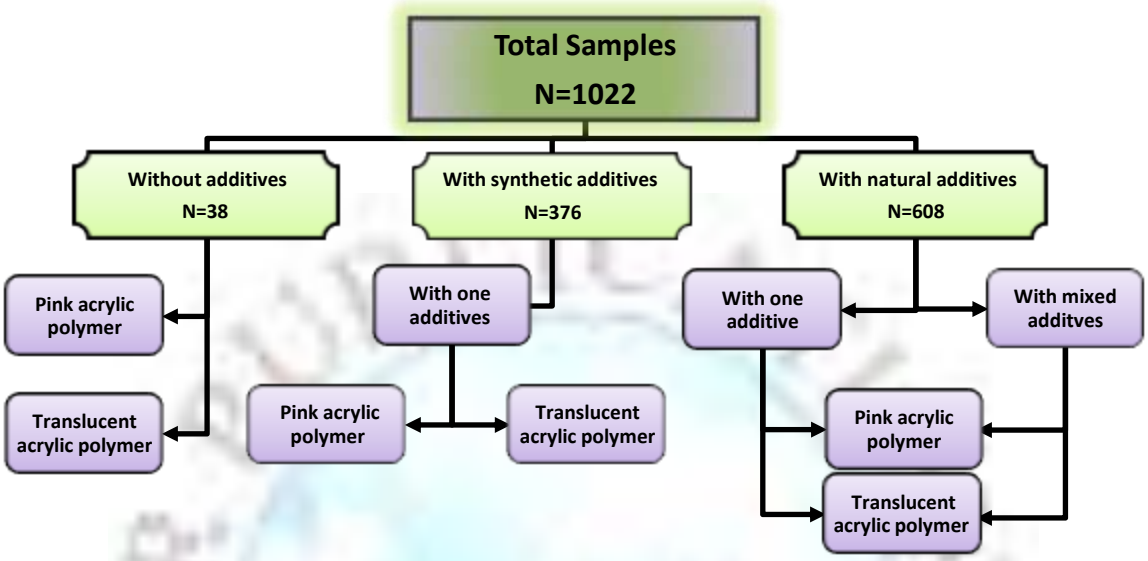

Figure (3.2): Experimental design of main study.

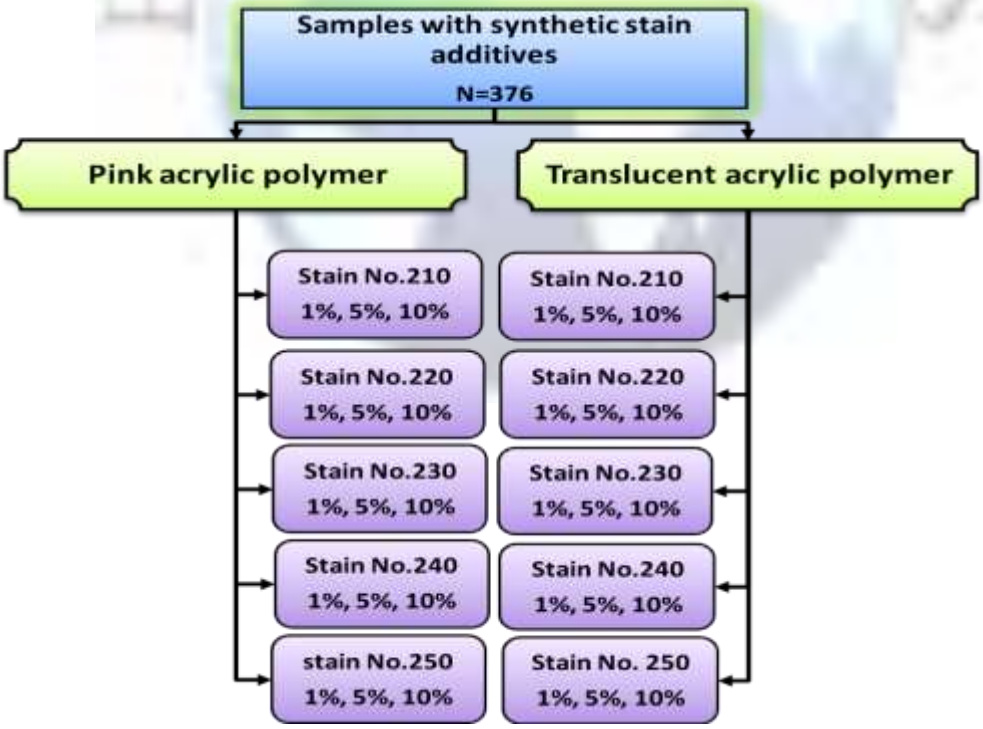

Figure (3.3): Samples with Vertex^{TM} synthetic acrylic stain additives.

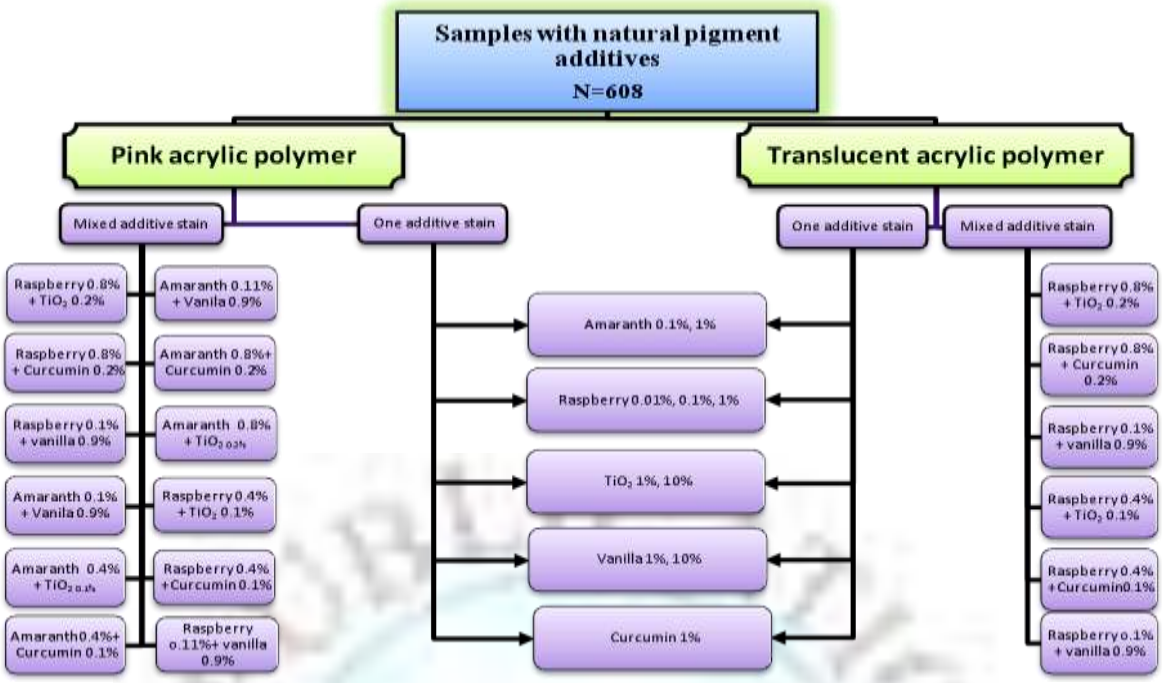

Figure (3.4): Samples with natural pigment additives.

3.3.2 Mixing of Polymer with Vertex™ Synthetic Acrylic Stain Additives

Mixing of the Vertex™ synthetic additives (stain No.210, stain No.220, stain No.230, stain No.240, stain No.250) were done with translucent acrylic powder and, pink acrylic powder in (1%, 5%, 10% wt/wt) in clean sealed plastic dry gar, and shaking thoroughly then sieving with a metal mesh size 100 µm to determine the particle size of powder and to be sure of mixing of stain particles with acrylic particles together.

3.3.3 Mixing of Polymer with Natural Pigments Additives

Grind the additives powder (Amaranth, Raspberry, Vanilla, Curcumin and TiO_2) to a very fine particles, then mixing translucent acrylic powder with Amaranth pigment that give the red color in different concentrations (0.1%,1%), with Raspberry in (0.01%, 0.1%, 1%), with Vanilla in (1%,

10%),with Curcumin in 1% that give the yellow grades and with TiO_2 in (1%, 10%) wt/wt that increase whiteness of samples in clean dry gar of the first group, and then with pink Vertex™ polymer as a second group, put in a sealed plastic container and shaking thoroughly then sieving by using sieve with a mesh size (100μm) for the following purposes:

- Estimation of particle size of each type additives was accomplished by assuring the additives particles size are not larger than the acrylic powder, using mesh sieves size 100μm.
- The sieving procedure was performed by mixing the acrylic powder thoroughly to ensure the proper dispersion of the additive particles through the acrylic powder polymer (Stafford *et al.*, 1980).

3.3.4 Preparation of Samples:

3.3.4.1 Preparation of the Molds

The specimens were prepared by cutting the Biostar (master model) according to the dimensions of the samples specified for each test as follows (Figure 3.5):

A. Color property measurement by the Vita Easyshade: The dimensions were (30x20x1.5) ±0.03mm (length, width and thickness respectively) (Hatim *et al.*, 2004).
B. Residual monomer test: The dimensions were (20x20x3) ±0.03mm (length, width and thickness respectively) (Azzarri *et al.*, 2003).
C. Indentation hardness and volumetric change test: The dimensions were (20x20x2.5) ±0.03mm (length, width and thickness respectively) (ADA Specification No. 12, 2002).
D. Water sorption and solubility test: The dimensions were (12x10x4) ±0.03mm (length, width and thickness respectively) (Podgórski, 2010).

E. FTIR test: The dimensions were (10x4x4) ±0.03mm (length, width and thickness respectively) (Urban *et al.*, 2007).

Figure (3.5): The Models of Biostar Used in the Preparation of the Samples. A: Color property measurement test sample. B: Residual monomer test sample. C: Indentation hardness and volumetric change test sample. D: Water sorption and solubility test sample. E: FTIR test sample.

3.3.4.2 Preparation of Translucent Acrylic Samples with Additives:

Two hundred and forty eight (120 with synthetic additives, and 128 with natural additives) samples preparation for color measurements were fabricated as mixing of the prepared powder (translucent heat cured acrylic polymer with one additive), as Figure (3.6):

- Amaranth (0.1%, 1%).
- Raspberry (0.01%, 0.1%, 1%).
- TiO_2 (1%, 10%).
- Vanilla (1%, 10%).
- Curcumin (1%).

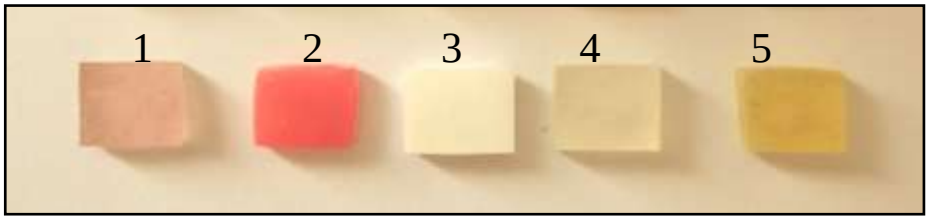

Figure (3.6): Samples of translucent heat cured acrylic with natural pigments in different concentrations. (1): Amaranth 0.1%, (2): Raspberry 1%, (3): TiO_2 1%, (4): Vanilla 10%, (5): Curcumin 1% wt/wt.

Other trails by mixing with two natural additives:

- Raspberry 0.4 % + TiO_2 0.1 %.
- Raspberry 0.8 % + TiO_2 0.2 %.
- Raspberry 0.1 % +Vanilla 0.9 %.
- Raspberry 0.11 % + Vanilla 0.9 %.
- Raspberry 0.4% + Curcumin 0.1 %.
- Raspberry 0.8 % + Curcumin 0.2 %.

Trails by using Vertex™ synthetic additives as Figure (3.7):

- Stain No.210 (1%, 5%, 10%).
- Stain No.220 (1%, 5%, 10%).
- Stain No.230 (1%, 5%, 10%).
- Stain No.240 (1%, 5%, 10%).
- Stain No.250 (1%, 5%, 10%).

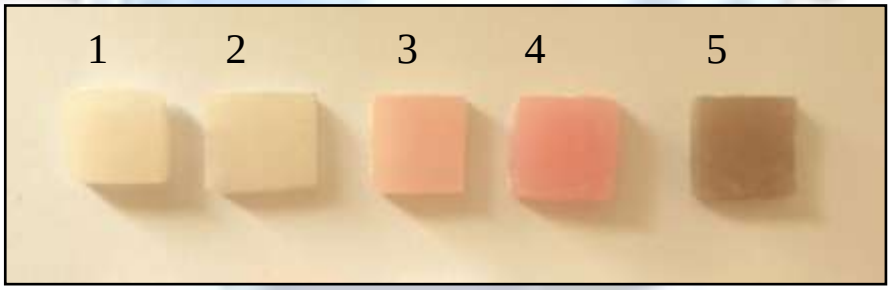

Figure (3.7): Samples of translucent heat cured acrylic with 10% wt/wt Vertex™ synthetic additives. (1): with synthetic stain No.210, (2): with synthetic stain No.220, (3): with synthetic stain No.230, (4): with synthetic stain No.240, (5): with synthetic stain No.250.

Mixing of powder with monomer was done according to the manufacturer instructions ratio (22g powder/10ml monomer), and cover the mixture and wait (30 minutes) to reach dough stage then apply it to mold and press to (200 µpa) pressure for (10 minutes), then curing in short cycle according to

manufacturer instructions (start with tap water and gradually increase the temperature until reach boiling degree and maintain at 100^{o}C for (30 minutes), bench cooling then open the flask and remove the samples.

3.3.4.3 Preparation of Pink Acrylic Samples with Additives

Three groups of total (736) samples preparation were fabricated as follows:

G1: Seven hundred and sixteen samples of color, indentation hardness, volumetric change, water sorption and solubility and residual monomer were prepared as mixing of the prepared powder (pink heat cured acrylic polymer) with one natural additive as Figure (3.8), with the followings concentration selected after doing several trails:

- Amaranth (0.1%, 1%).
- Raspberry (0.01%, 0.1%, 1%).
- TiO_2 (1%, 10%).
- Vanilla (1%, 10%).
- Curcumin 1%.

Other trails by mixing with two natural additives:

- Amaranth 0.1 % + Vanilla 0.9 %.
- Amaranth 0.11 % + Vanilla 0.9 %.
- Amaranth 0.4 % + Curcumin 0.1 %.
- Amaranth 0.8 % + Curcumin 0.2 %.
- Amaranth 0.4 % + TiO_2 0.1 %.
- Amaranth 0.8 % + TiO_2 0.2 %.
- Raspberry 0.1 % + Vanilla 0.9 %.
- Raspberry 0.11 % + Vanilla 0.9 %.

- Raspberry 0.4 % + Curcumin 0.1 %.
- Raspberry 0.8 % + Curcumin 0.2 %.
- Raspberry 0.4 % + TiO_2 0.1 %.
- Raspberry 0.8 % + TiO_2 0.2 %.

Trails by using Vertex™ synthetic additives (Figure 3.9):

- Stain No.210 (1%, 5%, 10%).
- Stain No.220 (1%, 5%, 10%).
- Stain No.230 (1%, 5%, 10%).
- Stain No.240 (1%, 5%, 10%).
- Stain No.250 (1%, 5%, 10%)

Figure (3.8): Samples of pink heat cured acrylic with natural pigments in different concentrations. (1): Amaranth 0.1%, (2): Raspberry 0.01%, (3): TiO_2 1%, (4): Vanilla 10%, (5): Curcumin 1% wt/wt.

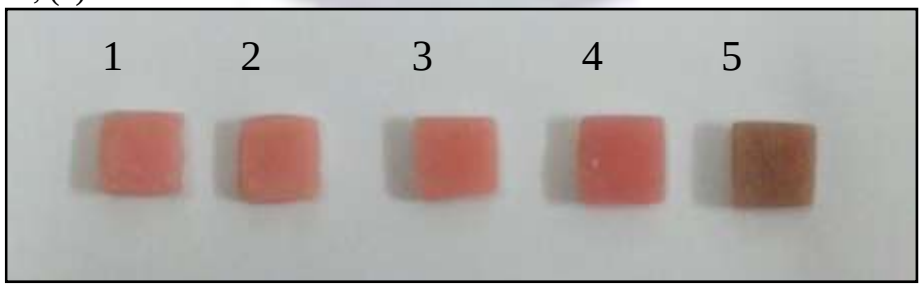

Figure (3.9): Samples of pink heat cured acrylic with Vertex™ synthetic stain 10% wt/wt additives. (1): with synthetic stain No.210, (2): with synthetic stain No.220, (3): with synthetic stain No.230, (4): with synthetic stain No.240, (5): with synthetic stain No.250.

Mixing of powder with monomer was done according to the manufacturer instructions ratio as mentioned in section (3.3.4.2).

G2: Nine samples for microscopic examination were prepared as mixing of the prepared powder with the Taha indicator* oil and examine it under reflecting light microscope (Rejab, 2002).

G3: Eleven samples for FTIR test fabricate samples as in (G1) and socking in distilled water for 48 hrs and remove it from water for 24hrs and grind the samples to obtain fine powder.

Total acrylic samples prepared (N=749) for the color measurement (248 samples of translucent acrylic with additives, 485 samples of pink acrylic with additives, 8 samples of translucent acrylic without additives, and 8 samples of pink acrylic without additives), taking (528) samples only and excluding the others that were resulting unacceptable hue (H) color value far away from the required in dental uses. It was difficult to determine the proper concentration of stain additives and tried to reach the desired colors, sixty eight different concentrations are prepared from both translucent and pink samples with additives (31 for translucent and, 37 for pink). High concentrations of additives were started and consequently decreased concentrations until reach the desired color values, too much samples were excluded from the other tests than color test in this study because there are no previous studies about the range of additive concentrations used to refer as guideline.

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*Taha Indicator: Is one of saturated cyclic hydrocarbons known as cycloalkanes which is relatively inert, ordinary do not react with most common acids, bases, oxidizing or reducing agents (Hart, 1987).

3.4 Tests Used in this Study

Following tests were used to examine some of the mechanical,chemical and physical properties of the samples:

1. Measurement of color property.
2. Indention hardness test.
3. Volumetric changes test.
4. Calculate of water sorption and solubility.
5. Measurement of residual monomer concentration.
6. FTIR test.
7. Microscopic examination test.

3.4.1 Measurement of Color Property

Samples were prepared as described in section (3.3) and storing acrylic samples in non ionized distilled water for 7 days at 37°C ±1 °C before testing. Assessment of color properties was performed by using Vita easyshade device (Vita Zahnfabrik, Germany) to measure color of the prepared acrylic samples, gingival color of twenty four humans and Vertex™ gingival shade guide.

All prepared color samples and Vertex™ gingival shade guide were measured with the same constant white background.

Color measurements of eight samples of each prepared type evaluated and the mean of L*, a* and b* values were calculated, measuring gingival color of healthy (12 male, 12 female), 24±1years old, 4th grade dentistry student in University of Mosul, in the anterior region attached gingiva (in midpoint between free gingiva and most deepest point of sulcus in central and lateral incisor regions about 2.5 mm apical to the crest of marginal gingiva) was measured (Huang *et al*., 2011). The tip of the measurement instrument was gently and surely adapted to the surface of the gingiva as showed in

Figure (3.10). This is because the deposits of pigments are more clear in this area, which may be confirmed by further research investigations (Oluwole and Elizabeth, 2010). The color values were measured in the ten tabs of the Vertex™ gingival shade guide (contain ten tabs color grades coded from 1, 2, to 10 shade), as shown in Figure (3.11).

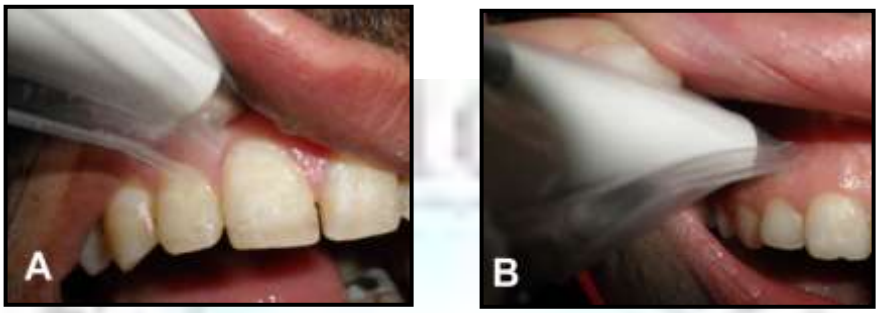

Figure (3.10): Measuring gingival color at (A); Central incisor region (B); Lateral incisor region.

Figure (3.11): Vertex™ gingival shade guide Measured with VITA Easyshade.

Measurement color matching: (CIE L*a*b*) color difference metrics were used for the performance analysis. Measurements were done by Vita Easyshade device to obtain the baseline L*, a*, b* values.

(CIE L*a*b*) color difference metrics were used for the performance analysis of the samples in the current study. The measured values of L*, a*, b* calculated consequently in the device and will appear in the screen.

The total color change (ΔE) between values was calculated for each pairs evaluated using the formula (Wee *et al.*, 2006; Anand *et al.*, 2007; and Cal *et al.*, 2007):

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad \ldots\ldots\ldots \quad (3\text{-}1)$$

$$\Delta E = [(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2]^{1/2} \quad \ldots\ldots\ldots \quad (3\text{-}2)$$

In principle, when no color difference detected after its exposure to the testing environment (ΔE=0) (Arthur *et al.*, 2004), (ΔE) of (3.7) or less is considered to be clinically acceptable in vitro study and of (6.8) or less is considered to be clinically acceptable in vivo study (Johnston and Kao, 1989).

Study of color of the natural pigment additives is a wide field due to the huge probability of the suggested concentration of the additive, specially there are limited studies related to this subject or related to it; like study of color of the human gingiva to correlate it with gingival shade guide.

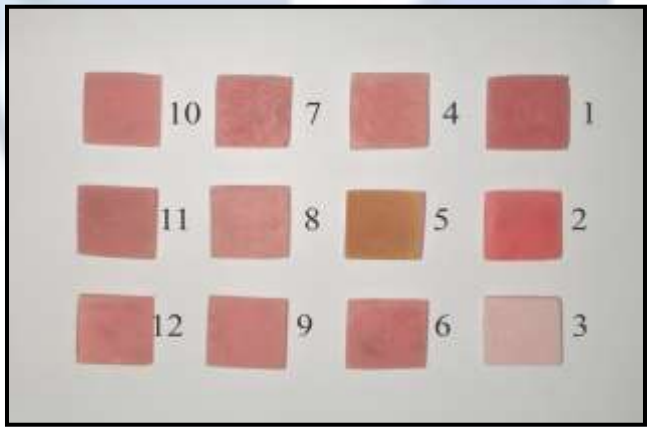

Figure (3.12): Samples of the selected groups of pink acrylic with natural and Vertex™ synthetic additives. (1): Amaranth 0.1% (G1), (2): Raspberry 0.01% (G2), (3): TiO_2 1% (G3), (4): Vanilla 10% (G4), (5): Curcumin 1% (G5), (6): stain No.240 1% (G6), (7): stain No.240 10% (G7), (8): stain No.220 5% (G8), (9): stain No.220 10% (G9), (10): stain No.210 5% (G10), (11): stain No.250 1% (G11), (12): control pink (without additives) (G12).

3.4.2 Indentation Hardness Test

Sixty Samples were prepared as described in section (3.3.4) and storing acrylic samples in non ionized distilled water for 48hrs at 37^{o}C ±1^{o}C before testing.

Using (Brook device) with 1/2 inch indenter as in Figure (3.13) with 100 kg application load applied to the samples surface (Issac, 1992).

Figure (3.13): Rockwell hardness tester (Brooks, Germany).

3.4.3 Volumetric Changes Test

Sixty samples were prepared as described in section (3.3.4). At two time intervals, immediately after curing samples and after 48hrs immersed in non ionized distilled water samples were assessed for its dimensional changes using electronic digital caliper. The dimensions of each sample were measured at three points for each measurement selected at least 2mm from the sample peripheries and the average of them was depended. Calculate V_1 (the volume [length x width x height] of each sample after removed from the flask) and

then immersed in distilled water for 48hrs at 37°C ±1°C then calculate the volume of each sample as V_2 (Abdul Razzak, 2010).

3.4.4 Calculate the Water Sorption and Solubility

Thirty six samples were prepared as described in section (3.3.2). The samples were dried in air at 37 °C until their weight was constant, and this result was recorded as (m_1). The specimens were then immersed in non ionized distilled water and maintained at 37°C for one week. After this time, the samples were removed, blotted to remove surface water, dried in air for 15 sec., and weighed the result was recorded as (m_2). After this weighing, the specimens were placed in the desiccator that contained anhydrous calcium chloride and dried at 37 °C until a final constant mass was obtained (m_3) The volumes of the specimens (V) were also measured. To calculate the water sorption (wsp) and solubility (wsl), the following equations were used (Podgórski, 2010):

$$wsp = m_2 - m_1/V \quad \ldots\ldots\ldots \quad (3\text{-}3)$$

$$wsl = m_2 - m_3/V \quad \ldots\ldots\ldots \quad (3\text{-}4)$$

3.4.5 Measurement of Residual Monomer Concentration

Sixty samples were prepared as described in section (3.3.4), all samples were introduced in a sealed glass container containing (10 ml) of distilled water at 37°C ±1°C, at appropriate time intervals (24hrs, 48hrs, 3rd day, 4th day, 5th day, 6th day and 7th day) the supernatants were removed and replaced by (10 ml) of fresh non ionized distilled water.

The time – dependence of the monomer concentration was followed by monitoring the amount of monomer present in the supernatant medium using a UV–Visible DUAL Spectrophotometer at (λ= 245 nm) (Figure 3.14) (Azzarri *et al.*, 2003). A linear calibration curve of methylmethacrylate (MMA)

concentration as a function of the absorbance at (245 nm) was obtained using MMA standard aqueous solutions (Figure 3.15). The results were expressed as a percent of released residual monomer mass with respect to the weight of the specimen (Azzarri *et al*., 2003).

Figure (3.14): Ultra Violet – Visible Spectrophotometer (DUAL 8 AUTO CELL uvs-2800 - USA)

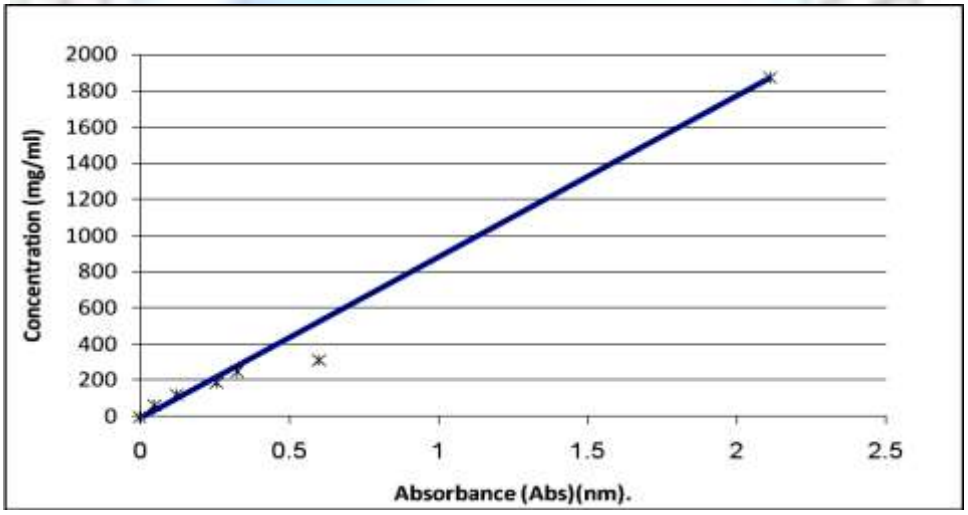

Figure (3.15): Linear calibration curve of Vertex™ heat cured acrylic resin material monomer (MMA).

3.4.6 FTIR Test

FTIR of the materials under investigation was determined by Fourier transform infrared spectroscopy (FTIR) samples of heat polymerizing resin

with dimension of (10x4x4)±0.03 mm (Urban *et al.*, 2007) were prepared and stored in non ionized distilled water at 37(±1)^{o}C for 48 hours. 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 (BRUKER TENSOR–27, Germany) as Figure (3.16) (Sarbu *et al.*, 2004). The FTIR spectra were carried by Bruker FTIR spectrophotometer in the Chemistry Department, College of Education Mosul University, to obtain the chart of the wavelength absorbed and that transmitted in the (500-4000 nm) region (Parikh, 1974).

Figure (3.16): Spectrophotometer (Fourier transform infrared spectroscopy).

3.4.7 Microscopic Examination

Testing procedure is by mixing about (1mg) of prepared powder as described in sections (3.3.2) and (3.3.3) on glass slide with one drop of Taha indicator, the mixture should not be too dense, then covered with cover slip, then examined and photographed under light microscope.

3.5 Statistical Analysis

Statistical analysis of color measurements was carried out using a special software designed by programmer in College of Computer Science & Mathematics/ Programming Engineering Section, in MATLAB (2010) for this study to calculate (ΔE) between every value with all others (in between for all measured values used in this study) and output appeared results of only the paired samples that (ΔE) ≤ 6.8.

Statistical analysis of other tests was made using (SPSS version 19) computer software; two tests were used to compare means. One Way Analysis of Variance (ANOVA) followed by Duncan's multiple range tests were used to compare between groups with indentation hardness test, volumetric change, water sorption and solubility tests and calculate of residual monomer conc.

T-test Paired samples also was used to compare between samples before and after 48hrs immersion in non ionized distilled water in volumetric change test.

CHAPTER FOUR

RESULTS

4.1 Color Property Test

4.1.1 Color Values of the Prepared Translucent Acrylic Samples According to L*a*b* System Graph.

In the L*a*b* system a color location is defined by the distances on the coordinates L, a, and b axes. Figures (4.1 and, 4.2) showed graphs of each group tristimuli L* , a* & b* values were analyzed by SPSS (version 19) computer software.

Three variables (L, a, b) are available for characterization of sample color, represents a tridimensional color space with three axes. (L) values of the prepared samples are plotted in Y axis, (a) values plotted in X axis and (b) values plotted in Z axis according to Lab graph system. Where the point (L.a.b) location as in Figures (4.1 and, 4.2) representation the exact point location of the sample color in relation to the 3 axes.

The letter (L) represents the measure of luminosity or clarity of a sample, and it is quantified according to a scale on which the perfect black has value of (L) equal to (zero), whereas the total white has value of (L) equal to (100). There are also two chromatic components, which represent variations in hue and chroma. The (a) axis is measured from red (a in positive) to green (a in negative), varying respectively. The (b) axis is measured from yellow (b in positive) to blue (b in negative). The coordinates (a) and (b) approach zero for neutral colors (white, gray) and increase in magnitude for more saturated and intense colors (Ishikawa-Nagai *et al.*, 2004; Luo, 2007).

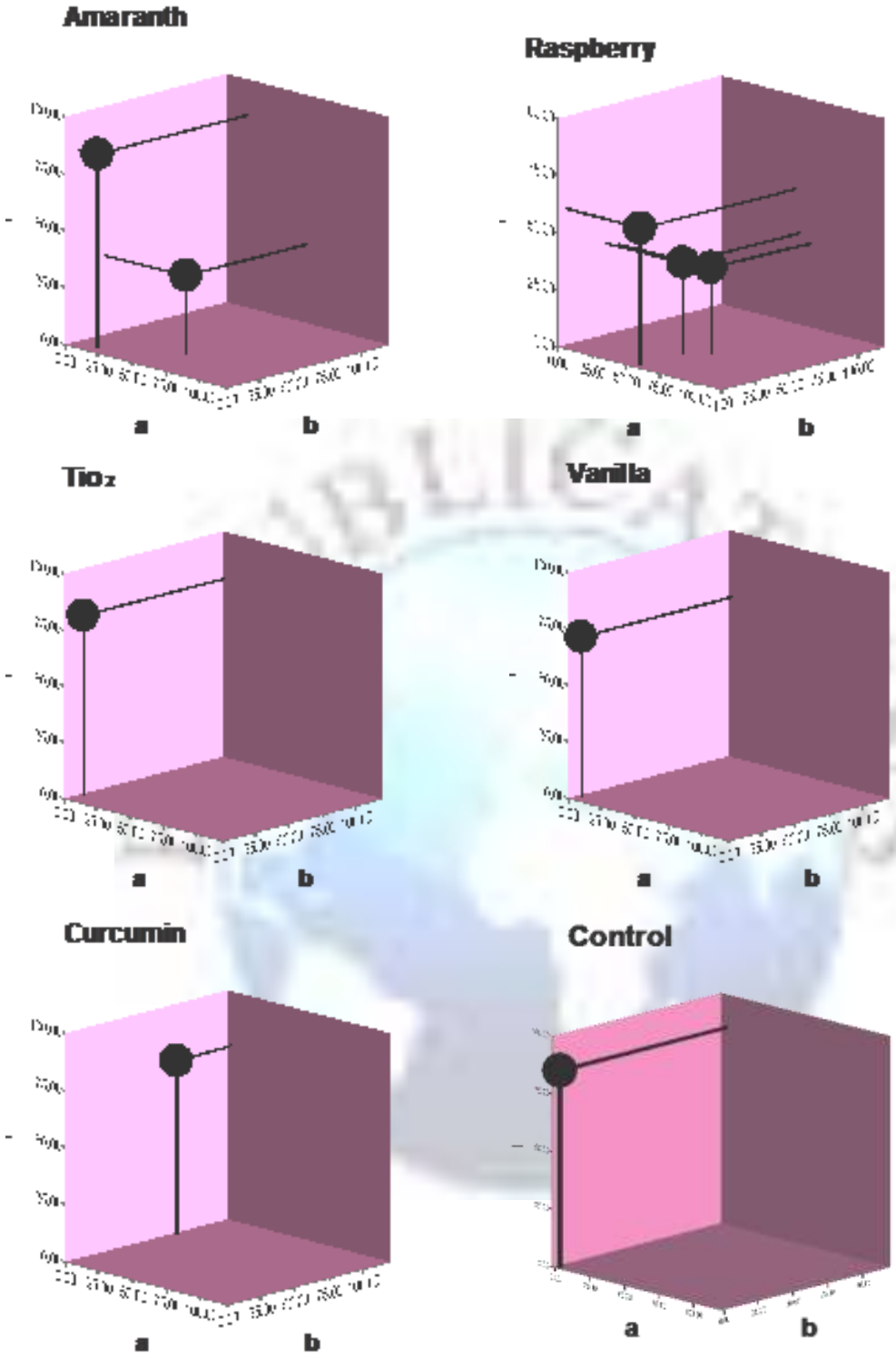

Figure (4.1): L* , a* & b* values of control and samples with natural pigment additives represented in Lab graph. Amaranth (0.1%, 1% wt/wt), Raspberry (0.01%, 0.1%, 1% wt/wt), TiO_2 (1%), Vanilla (10%), Curcumin (1%wt/wt), Control translucent without additives.

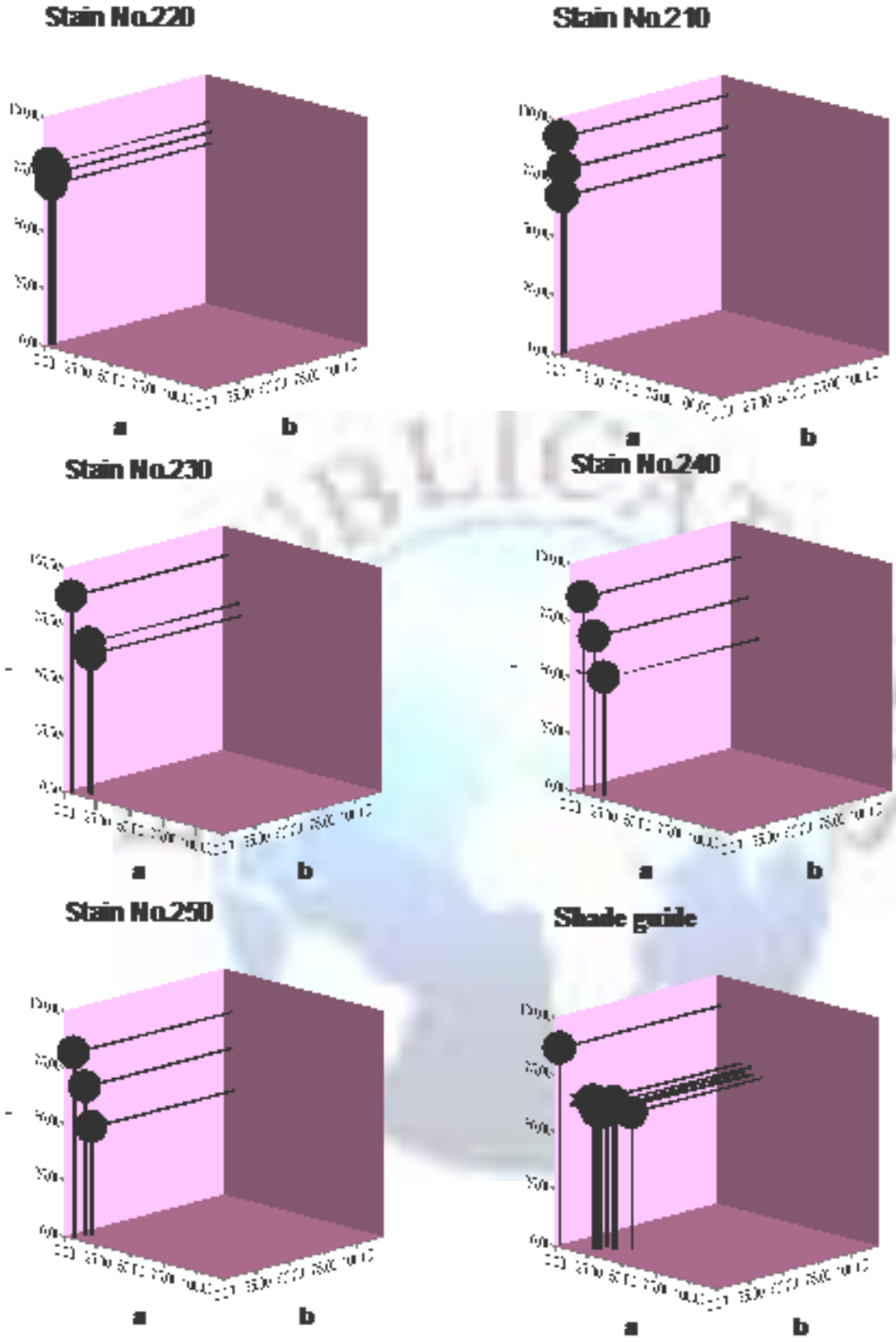

Figure (4.2): L* , a* & b* values of Vertex™ synthetic additive samples and Vertex™ shade guide represented in Lab graph. Synthetic stain [No.210 (10% wt/wt), No.220 (10% wt/wt), No.230 (10% wt/wt), No.240 (10% wt/wt), No.250 (10% wt/wt)] and Vertex artificial gingival shade guide.

Table (4.1): Effects of natural and synthetic additives on color values of the samples in relation to control.

Color values	Natural additives	Synthetic additives
Lightness (L)	All decrease lightness except vanilla	All decrease lightness
Redness (a)	Amaranth, Raspberry and Vanilla increase redness TiO_2 and Curcumin decrease redness	All increase redness
Yellowness (b)	All increase yellowness, curcumin is maximum	All increase yellowness

4.1.2 Color Values of the Prepared Pink Acrylic Samples, Measured Patient's Gingiva and Gingival Shade Guide.

The values of the color measurement of oral cavity gingival part of (24) patients are listed in Tables (4.2 and, 4.3). That maximum value of (L=76.2) and the minimum value (L=38.9), for value (a=36.0) as maximum and (a=3.0) as minimum, for value (b=50.1) as maximum and, (b=13.6) as minimum.

Table (4.2): The mean values of color measurement of the female patients' gingiva.

Patients	Gender	Area	L	a	b
1	**f**	Region a	58.2	19.5	25.6
		Region b	53.7	28.0	27.0
2	**f**	Region a	50.8	27.2	21.6
		Region b	59.7	27.6	26.9
3	**f**	Region a	38.9	26.7	25.3
		Region b	44.2	25.1	29.6
4	**f**	Region a	57.5	16.5	25.6
		Region b	67.0	10.6	28.3
5	**f**	Region a	64.7	10.2	25.9
		Region b	45.4	29.1	19.3
6	**f**	Region a	76.2	3.0	25.8
		Region b	50.4	26.7	15.2
7	**f**	Region a	52.9	19.0	23.8
		Region b	48.9	23.2	13.6
8	**f**	Region a	69.5	7.2	50.1
		Region b	5.8	19.8	28.4
9	**f**	Region a	50.8	17.1	25.3
		Region b	51.7	17.6	36.3
10	**f**	Region a	52.9	16.3	21.8
		Region b	55.0	19.5	31.9
11	**f**	Region a	47.4	25.2	21.7
		Region b	55.6	16.2	38.5
12	**f**	Region a	53.5	24.1	28.0
		Region b	71.0	12.9	31.6

L=degree of lightness, a=degree of redness and greenness, b=degree of yellowness and blueness, f=female, Region a= central incisor region, Region b = lateral incisor region.

Table (4.3): The mean values of color measurement of the male patients' gingiva.

Patients	Gender	Area	L	a	b
13	**m**	Region a	42.6	36.0	20.2
		Region b	57.2	14.8	27.2
14	**m**	Region a	59.3	16.7	23.5
		Region b	53.7	25.8	24.8
15	**m**	Region a	40.2	33.7	17.8
		Region b	54.4	22.7	24.7
16	**m**	Region a	61.7	16.0	28.7
		Region b	46.2	31.6	21.3
17	**m**	Region a	56.9	17.1	28.4
		Region b	63.4	15.5	29.6
18	**m**	Region a	66.0	12.1	31.2
		Region b	54.7	22.4	24.2
19	**m**	Region a	60.4	15.2	35.5
		Region b	55.1	15.2	35.5
20	**m**	Region a	58.6	16.3	25.9
		Region b	52.4	26.5	22.4
21	**m**	Region a	54.8	19.1	28.8
		Region b	57.2	15.2	27.2
22	**m**	Region a	74.0	6.6	34.9
		Region b	60.5	16.6	28.2
23	**m**	Region a	53.8	22.4	30.4
		Region b	57.2	17.3	36.8
24	**m**	Region a	52.3	20.7	25.5
		Region b	57.3	18.2	29.6

L=degree of lightness, a=degree of redness and greenness, b=degree of yellowness and blueness, m=male, Region a= central incisor region, Region b = lateral incisor region.

Results of the color matching between the control group (G12) and groups with Vanilla additive (G4), group with Amaranth additive (G1) are shown in Table (4.4). Color matching results between the human gingival colors and samples with natural and synthetic additives are shown in Tables (4.5 – 4.7).

Table (4.4) : Color values mean of control pink group and group with Amaranth and Vanilla additives.

Groups	L	a	b	C	H
Samples without additives (control) (G12)	51.80	28.35	12.35	30.91	23.52
Samples with Amaranth 0.1% additive (G1)	41.90	35.98	15.98	39.40	23.88
Samples with Vanilla 10% additive (G4)	53.44	27.28	12.04	30.02	23.78

L; degree of lightness, a; degree of redness and greenness, b; degree of yellowness and blueness, C; chroma (saturation), H; hue color value.

Table (4.5): Natural and synthetic coloring additives matched with human gingival color.

Matched groups			ΔE	Acceptance
Samples with Vanilla additives 10% (G4)	vs.	6fb	4.42	In vivo
Samples with synthetic stain No.240 10% (G7)	vs.	6fb	5.49	In vivo
Samples with Amaranth additive in 0.1% (G1)	vs.	13ma	5.01	In vivo
Samples with mixed Amaranth 0.1% + Vanilla	vs.	13ma	3.30	In vivo and in vitro

6fb; female patient's gingival color in lateral incisor region, 13ma; male patient's gingival color in central incisor region.
(ΔE)=0 no color change, (ΔE) ≤ 3.7 accepted in vitro color change, (ΔE) ≤ 6.8 accepted in vivo color change.

Table (4.6): Different concentrations of the synthetic color additives matched the same human gingival color.

Matched groups			ΔE	Acceptance
Samples with stain No. 230 5%	vs.	6fb	6.017	In vivo
Samples with stain No. 230 10%	vs.	6fb	6.12	In vivo
Samples with stain No. 240 1% (G6)	vs.	6fb	5.47	In vivo
Samples with stain No. 240 10% (G7)	vs.	6fb	5.49	In vivo
Samples with stain No. 250 1% (G11)	vs.	7fb	4.96	In vivo
Samples with stain No. 250 5%	vs.	7fb	5.52	In vivo

6fb; female patient's gingival color in lateral incisor region, 7fb; female patient's gingival color in lateral incisor region.
(ΔE)=0 no color change, (ΔE) ≤ 3.7 accepted in vitro color change, (ΔE) ≤ 6.8 accepted in vivo color change.

Table (4.7): Different colors of the synthetic additives matched the same human gingival color.

Matched groups			ΔE	Acceptance
Samples with stain No. 220 1%	vs.	6fb	5.30	In vivo
Samples with stain No. 230 1%	vs.	6fb	5.39	In vivo
Samples with stain No. 240 1% (G6)	vs.	6fb	5.40	In vivo

6fb; female patient's gingival color in lateral incisor region.
(ΔE)=0 no color change, (ΔE) ≤ 3.7 accepted in vitro color change, (ΔE) ≤ 6.8 accepted in vivo color change.

Table (4.8) show the number of matching between gingival color and the natural, synthetic stain, artificial gingival shade guide.

Table (4.8): Numbers of matching between gingival color and the natural, synthetic stain, artificial gingival shade guide.

Matched groups		Only matched with		ΔE	Acceptance
Synthetic stain 1%, 5%, 10%	No. : 210, 220, 230, 240,250	Vs.	6fb 7fb	5.30 - 6.12	In vivo
Artificial gingival shade guide	Tabs: 1,2,3,4,5, 6,7,8,9,10	Vs.	2fb	6.49	In vivo
Natural stains	Vanilla (1%, 10%)	Vs.	6fb 7fb 13ma 15ma	3.39 - 6.30	In vivo and In vitro
	Vanilla mixed with Amaranth in 0.09%	Vs.	13ma 15ma	3.39 4.96	In vivo, in vitro In vitro
	Amaranth (0.1%)	Vs.	15ma	3.42	In vivo and In vitro
	Amaranth mixed with Vanilla in 0.01%	Vs.	13ma 15ma	3.39 4.96	In vivo, in vitro In vitro

6fb; female patient's gingival color in lateral incisor region, 7fb; female patient's gingival color in lateral incisor region, 2fb; female patient's gingival color in lateral incisor region, 13ma; male patient's gingival color in central incisor region, 15ma; male patient's gingival color in central incisor region.

Selection only twelve groups among all prepared groups that matched by hue values as shown in Table (4.9) to continue other tests.

Table (4.9): Mean of hue value (the main color) of the chosen some groups to continue the other tests.

	Samples	**H**
G 1	Samples with Amaranth in 0.1% conc	23.88
G 2	Samples with Raspberry in 0.01% conc.	20.86
G 3	Samples with TiO_2 in 1% conc	35.40
G 4	Samples with Vanilla in 10% conc.	23.78
G 5	Samples with Curcumin in 1% conc.	65.80
G 6	Samples with synthetic stain No.240 in 1% conc	28.82
G 7	Samples with synthetic stain No.240 in 10% conc	22.16
G 8	Samples with synthetic stain No.220 in 10% conc	22.38
G 9	Samples with synthetic stain No.220 in 5% conc.	23.32
G 10	Samples with synthetic stain No.210 in 5% conc	21.34
G 11	Samples with synthetic stain No.250 in 1% conc	21.60
G 12	Control pink samples (without additive).	23.52

H; mean of hue value.

4.2 Indentation Hardness Test

The means, standard deviations of samples with and without additives were shown in Figure (4.3).

The One Way analysis of variance (ANOVA) was shown in Table (4.10) that used Rockwell hardness test type with 1/2 inch indenter, scale M, load 100Kg. There were significant differences at ($P<0.05$) in indentation hardness

test between control pink group (samples without additives) (G12) and groups with both natural and Vertex™ synthetic stain additives.

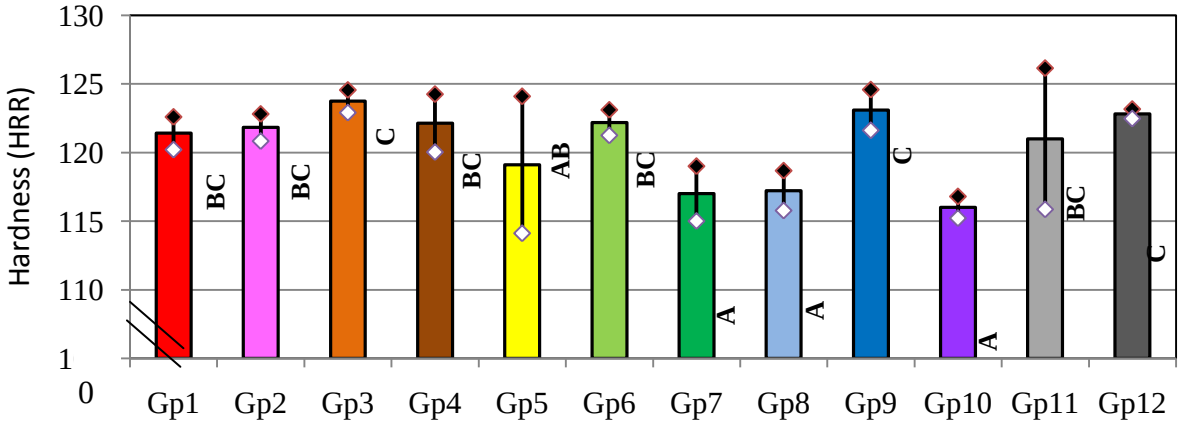

Figure (4.3): Mean, standard deviation, and Duncan's multiple range test for indentation harness test. G1; samples with Amaranth in 0.1% conc., G2; samples with Raspberry in 0.01% conc., G3; samples with TiO_2 in 1% conc., G4; samples with Vanilla in 10% conc., G5; samples with Curcumin in 1% conc, G6; samples with synthetic stain No.240 in 1% conc., G7; samples with synthetic stain No.240 in 10% conc., G8; samples with synthetic stain No.220 in 10% conc., G9; samples with synthetic stain No.220 in 5% conc., G10; samples with synthetic stain No.210 in 5% conc., G11; samples with synthetic stain No.250 in 1% conc., G12; control pink samples without additive.
*different letters means significant differences for Duncan's multiple range tests.

Table (4.10): The One Way ANOVA, for indentation hardness test of the samples (with and without additives).

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	379.690	11	34.517	6.017	0.000*
Within Groups	275.341	48	5.736		
Total	655.031	59			

* Significant difference at $p<0.05$. df: Degree of freedom.

Duncan's multiple range test showed that only groups (G5; samples with Curcumin in 1% conc., G7; samples with synthetic stain No.240 in 10% conc., G8; samples with synthetic stain No.220 in 10% conc. and G10; samples with synthetic stain No.210 in 5% conc.) significant decrease in indentation hardness from the control group (G12; pink samples without additives), while all other tested groups showed no significant differences at ($p<0.05$) in indentation hardness from the control group (G12).

4.3 Volumetric Change Test

The means and standard deviations of the samples' volumes (mm^3) after two days immersion in non ionized distilled water for samples (with and without additives) were shown in Figure (4.4).

The One Way (ANOVA) multiple comparison test between the samples' volume after two days immersed in non ionized distilled water was shown in Table (4.11). It is obvious from the analysis that there were no significant differences at ($p<0.05$) in volumetric change between groups of samples with additives and control groups (G12; pink samples without additives). Mean difference and percentage of samples volume before and after two days immersion in non ionized distilled water was shown in Table (4.11).

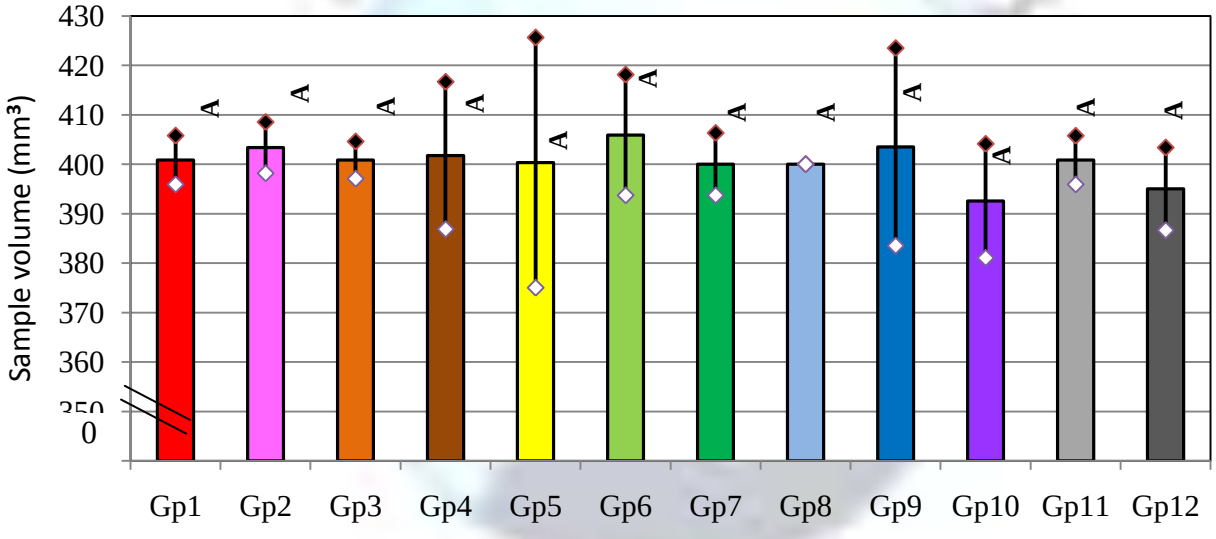

Figure (4.4): Mean, standard deviation, and Duncan's multiple range tests for volumetric change test for samples after two days immersion. G1; samples with Amaranth in 0.1% conc., G2; samples with Raspberry in 0.01% conc., G3; samples with TiO_2 in 1% conc., G4; samples with Vanilla in 10% conc., G5; samples with Curcumin in 1% conc, G6; samples with synthetic stain No.240 in 1% conc., G7; samples with synthetic stain No.240 in 10% conc., G8; samples with synthetic stain No.220 in 10% conc., G9; samples with synthetic stain No.220 in 5% conc., G10; samples with synthetic stain No.210 in 5% conc., G11; samples with synthetic stain No.250 in 1% conc., G12; control pink samples without additive.
*different letters means significant differences for Duncan's multiple range tests.

Table (4.11): One Way ANOVA, test for sample volume means.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	850.241	11	77.295	0.532	0.874
Within Groups	8716.357	60	145.273		
Total	9566.598	71			

df: Degree of freedom.

Duncan's multiple range test showed that, all the groups showed no significant differences at ($p<0.05$) from the control group (G12; pink samples without additives).

Table (4.12): Mean difference and percentages of prepared samples volume before and after two days immersion in non ionized distilled water.

Groups	Difference (mm^3) After-Before	Accuracy %
G1	**0**	**0.0 %**
G2	**3**	**0.03 %**
G3	**0**	**0.00 %**
G4	**1**	**0.01 %**
G5	**0**	**0.00 %**
G6	**5**	**0.05 %**
G7	**0**	**0.00 %**
G8	**0**	**0.00 %**
G9	**3**	**0.03 %**
G10	**-8**	**-0.08 %**
G11	**0**	**0.00 %**
G12	**-5**	**-0.05 %**

G1; samples with Amaranth in 0.1% conc., G2; samples with Raspberry in 0.01% conc., G3; samples with TiO_2 in 1% conc., G4; samples with Vanilla in 10% conc., G5; samples with Curcumin in 1% conc, G6; samples with synthetic stain No.240 in 1% conc., G7; samples with synthetic stain No.240 in 10% conc., G8; samples with synthetic stain No.220 in 10% conc., G9; samples with synthetic stain No.220 in 5% conc., G10; samples with synthetic stain No.210 in 5% conc., G11; samples with synthetic stain No.250 in 1% conc., G12; control pink samples without additive.

Paired samples T-test was performed on samples before immersion in non ionized distilled water comparing volume means of samples at period of two days immersion as shown in Table (4.13).

Table (4.13) Paired sample T-test for volumetric changes of samples before and after two days periods immersion in non ionized distilled water.

Group			T	df	p-value
G1	Pair 1	Before – After	-.418-	5	0.693
G2	Pair 1	Before – After	-1.581-	5	0.175
G3	Pair 1	Before – After	-.542-	5	0.611
G4	Pair 1	Before – After	-.289-	5	0.784
G5	Pair 1	Before – After	-.032-	5	0.976
G6	Pair 1	Before – After	-1.187-	5	0.288
G7	Pair 1	Before – After	-.004-	5	0.997
G9	Pair 1	Before – After	-.429-	5	0.686
G10	Pair 1	Before – After	1.575	5	0.176
G11	Pair 1	Before – After	-.415-	5	0.695
G12	Pair 1	Before – After	1.464	5	0.203

G1; samples with Amaranth in 0.1% conc., G2; samples with Raspberry in 0.01% conc., G3; samples with TiO_2 in 1% conc., G4; samples with Vanilla in 10% conc., G5; samples with Curcumin in 1% conc, G6; samples with synthetic stain No.240 in 1% conc., G7; samples with synthetic stain No.240 in 10% conc., G8; samples with synthetic stain No.220 in 10% conc., G9; samples with synthetic stain No.220 in 5% conc., G10; samples with synthetic stain No.210 in 5% conc., G11; samples with synthetic stain No.250 in 1% conc., G12; control pink samples without additive.

Paired sample T-test was done between samples volume means for all groups to find the differences before immersion and after two days immersion in non ionized distilled water, but there was no significant difference between two periods.

4.4 Measurement of Water Sorption

The means, standard deviations of samples with and without additives were shown in Figure (4.5).

The One Way (ANOVA) was shown in Table (4.14). There were significant differences ($P<0.05$) in measurement of water sorption for groups with additives and control group (G12; pink samples without additives).

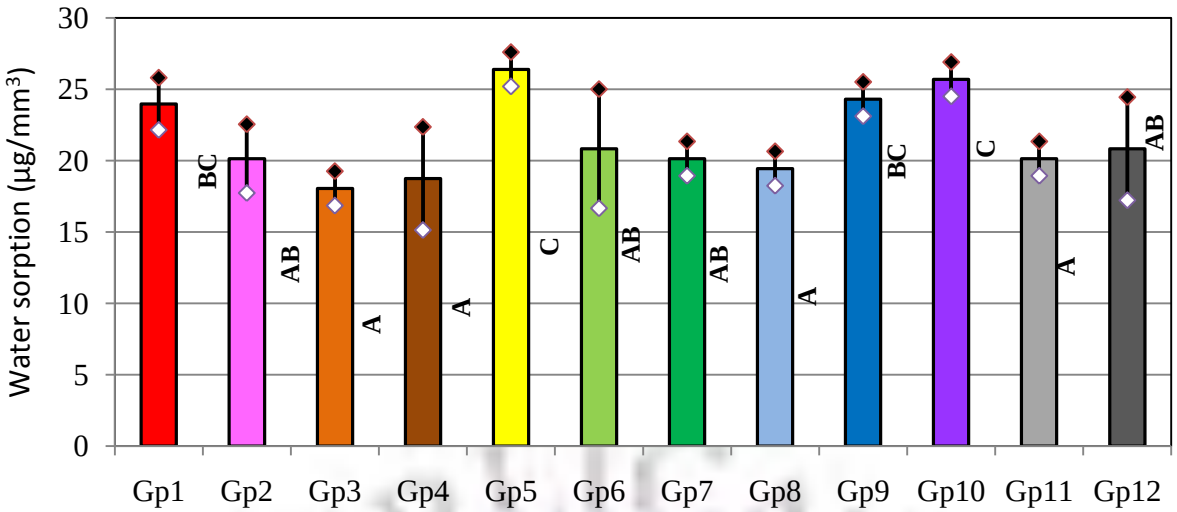

Figure (4.5): Mean, standard deviation, and Duncan's multiple range tests for measurement of water sorption. G1; samples with Amaranth in 0.1% conc., G2; samples with Raspberry in 0.01% conc., G3; samples with TiO_2 in 1% conc., G4; samples with Vanilla in 10% conc., G5; samples with Curcumin in 1% conc, G6; samples with synthetic stain No.240 in 1% conc., G7; samples with synthetic stain No.240 in 10% conc., G8; samples with synthetic stain No.220 in 10% conc., G9; samples with synthetic stain No.220 in 5% conc., G10; samples with synthetic stain No.210 in 5% conc., G11; samples with synthetic stain No.250 in 1% conc., G12; control pink samples without additive.
*different letters means significant differences for Duncan's multiple range tests.

Table (4.14): One Way ANOVA, test for measurement of water sorption.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	256.615	11	23.329	4.468	0.001*
Within Groups	125.322	24	5.222		
Total	381.937	35			

* Significant difference at $p<0.05$. df: Degree of freedom.

Duncan's multiple range test showed that only groups (G5; samples with Curcumin in 1% conc. And G10; samples with synthetic stain No.210 in 5% conc.) significant increase in water sorption from the control group (G12; pink samples without additives), while all the other tested groups showed no significant differences at ($p<0.05$).

4.5 Measurement of Water Solubility

The means, standard deviations of samples with and without additives were shown in Figure (4.6).

The One Way (ANOVA) was shown in Table (4.15). There were significant differences (P<0.05) in measurement of water solubility for groups with additives and control group (G12; pink samples without additives).

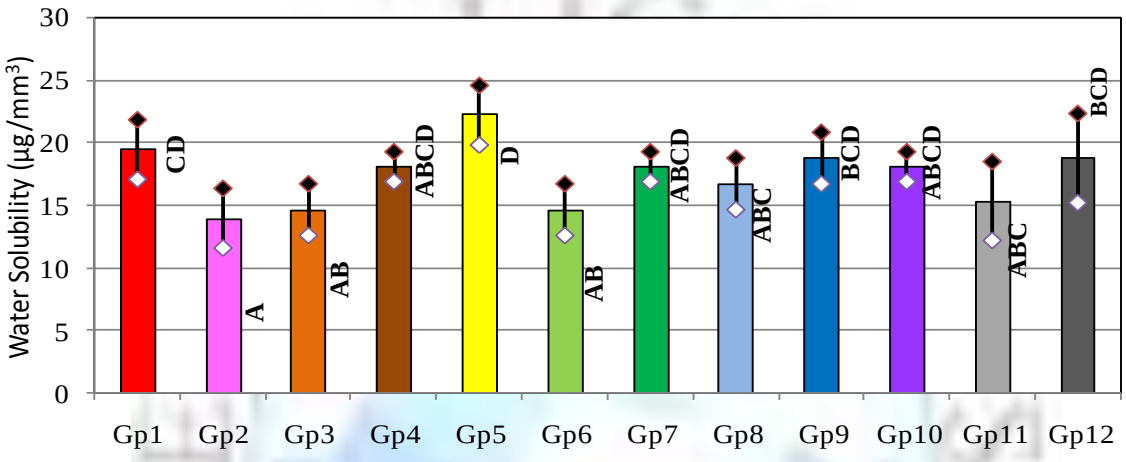

Figure (4.6): Mean, standard deviation, and Duncan's multiple range test for measurement of water solubility. G1; samples with Amaranth in 0.1% conc., G2; samples with Raspberry in 0.01% conc., G3; samples with TiO_2 in 1% conc., G4; samples with Vanilla in 10% conc., G5; samples with Curcumin in 1% conc, G6; samples with synthetic stain No.240 in 1% conc., G7; samples with synthetic stain No.240 in 10% conc., G8; samples with synthetic stain No.220 in 10% conc., G9; samples with synthetic stain No.220 in 5% conc., G10; samples with synthetic stain No.210 in 5% conc., G11; samples with synthetic stain No.250 in 1% conc., G12; pink control samples without additive.
*different letters means significant differences for Duncan's multiple range tests.

Table (4.15): One Way ANOVA, test for measurement of water solubility.

	Sum of Squares	Df	Mean Square	F	p-value
Between Groups	196.757	11	17.887	3.450	0.005*
Within Groups	124.425	24	5.184		
Total	321.182	35			

* Significant difference at p<0.05. df: Degree of freedom.

Duncan's multiple range test showed that only group (G2; samples with Raspberry in 0.01% conc.,) significant decrease in water solubility from the control group (G12; pink samples without additives), while all the other tested groups showed no significant differences at ($p<0.05$).

4.6 Residual Monomer Release Test

The amount of released monomer versus release period of time (in days) for the prepared samples without additives and with additives after storage in non ionized distilled water for seven days was evaluated every 24hrs. Residual monomer everyday release was measured in (µg) for seven days (Figure 4.7).

One Way (ANOVA) for residual monomer amount of samples showed that there was significance at ($p\leq0.05$) for all samples except (G8) synthetic™ stain additives No. 220 (10%) samples that was no significance at ($p \leq 0.05$) (Table 4.16).

Table (4.16): One Way ANOVA for residual monomer amount.

Group		Summation of Squares	df	Mean Square	F	Sig.
G1	Between Groups	31697.143	6	5282.857	36.582	0.000*
	Within Groups	4043.500	28	144.411		
	Total	35740.643	34			
G2	Between Groups	13844.186	6	2307.364	5.784	0.001*
	Within Groups	11170.600	28	398.950		
	Total	25014.786	34			
G3	Between Groups	32339.786	6	5389.964	17.813	0.000*
	Within Groups	8472.600	28	302.593		
	Total	40812.386	34			
G4	Between Groups	31233.543	6	5205.590	113.147	0.000*
	Within Groups	1288.200	28	46.007		
	Total	32521.743	34			

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G6	Between Groups	4080.686	6	680.114	15.558	0.000*
	Within Groups	1224.000	28	43.714		
	Total	5304.686	34			
G7	Between Groups	43736.671	6	7289.445	75.712	0.000*
	Within Groups	2695.800	28	96.279		
	Total	46432.471	34			
G8	Between Groups	3336.743	6	556.124	1.393	0.252
	Within Groups	11174.500	28	399.089		
	Total	14511.243	34			
G9	Between Groups	44278.471	6	7379.745	14.096	0.000*
	Within Groups	14659.100	28	523.539		
	Total	58937.571	34			
G10	Between Groups	4388.571	6	731.429	5.901	0.000*
	Within Groups	3470.500	28	123.946		
	Total	7859.071	34			
G11	Between Groups	56481.600	6	9413.600	10.083	0.000*
	Within Groups	26141.300	28	933.618		
	Total	82622.900	34			
G12	Between Groups	3756.386	6	626.064	7.216	0.000*
	Within Groups	2429.400	28	86.764		
	Total	6185.786	34			

* Significant difference at $p<0.05$. df: Degree of freedom.

G1; samples with Amaranth in 0.1% conc., G2; samples with Raspberry in 0.01% conc., G3; samples with TiO_2 in 1% conc., G4; samples with Vanilla in 10% conc., G6; samples with synthetic stain No.240 in 1% conc., G7; samples with synthetic stain No.240 in 10% conc., G8; samples with synthetic stain No.220 in 10% conc., G9; samples with synthetic stain No.220 in 5% conc., G10; samples with synthetic stain No.210 in 5% conc., G11; samples with synthetic stain No.250 in 1% conc., G12; control pink samples without additive.

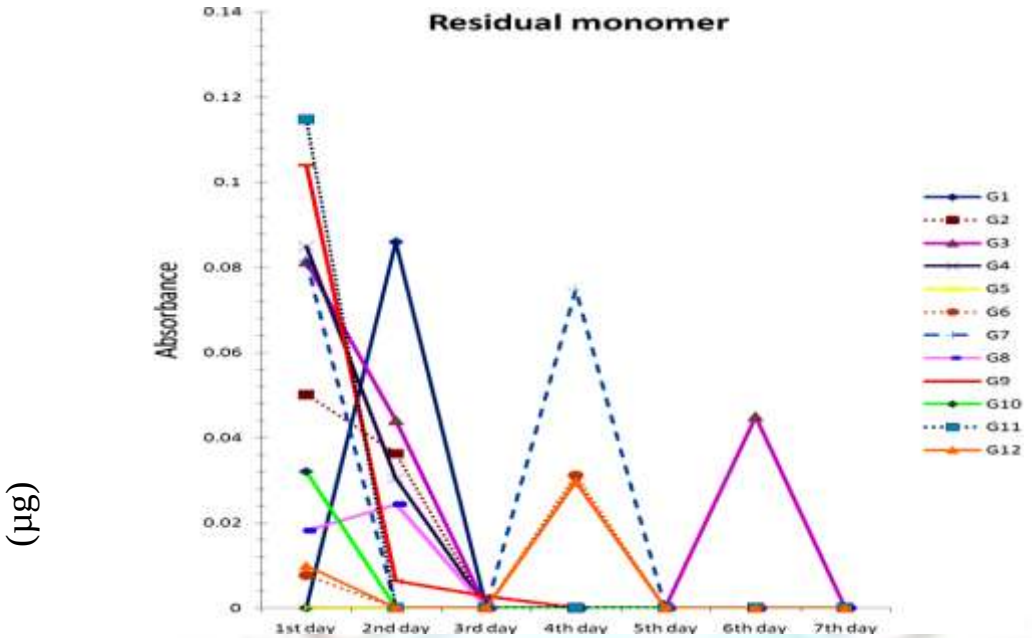


Figure (4.7): Daily residual monomer release of the prepared samples over seven days. G1; samples with Amaranth in 0.1% conc., G2; samples with Raspberry in 0.01% conc., G3; samples with TiO_2 in 1% conc., G4; samples with Vanilla in 10% conc., G5; samples with Curcumin in 1% conc, G6; samples with synthetic stain No.240 in 1% conc., G7; samples with synthetic stain No.240 in 10% conc., G8; samples with synthetic stain No.220 in 10% conc., G9; samples with synthetic stain No.220 in 5% conc., G10; samples with synthetic stain No.210 in 5% conc., G11; samples with synthetic stain No.250 in 1% conc., G12; control pink samples without additive.

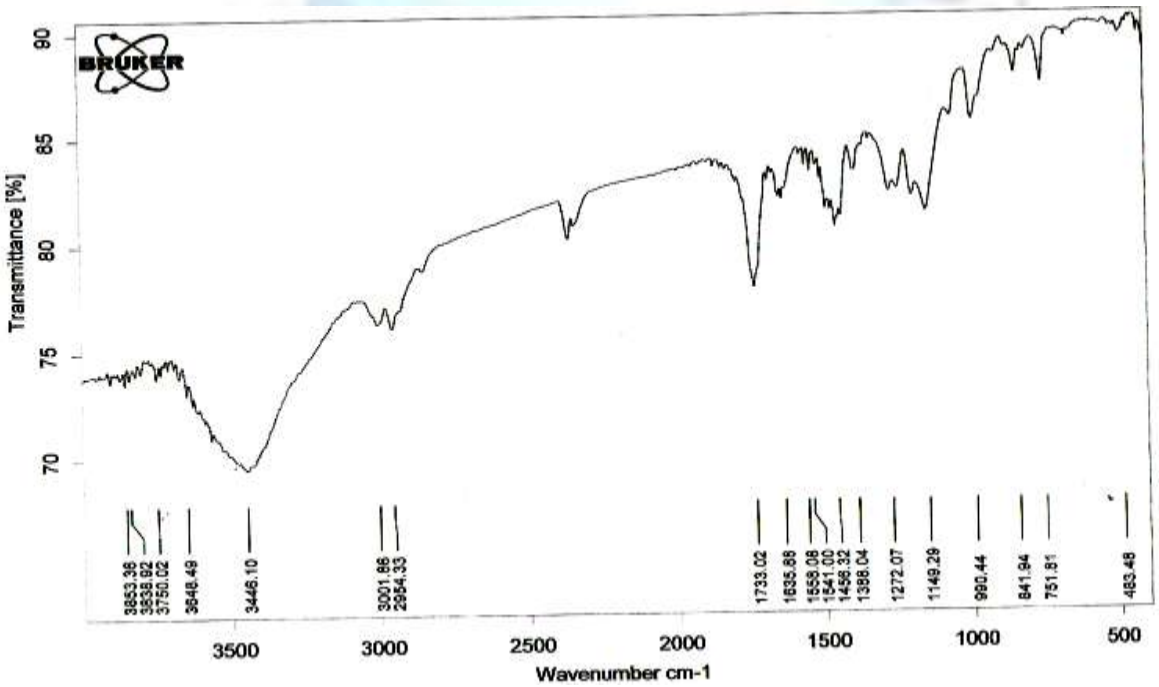
4.7 FTIR Test

In the IR charts, two important absorbance peaks appeared (the absorbance of the C=C bond 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}) (BAHL and Bahl, 2010). Infrared spectrometer chart of the monomer (methyl methacrylate) control group were shown in Figure (4.8).

Vanillin spectrum was examined between 1500 and 4000 cm^{-1}. There are (6) major peaks in this region. O−H stretch due to the OH group on the ring−H ring hydrogen stretch, C−H3 C−H stretch in the methoxy (O-CH3) group, HC=O C=O stretch in the aldehyde group, C=C two peaks due to the ring C=C stretch (BAHL and Bahl, 2010).

The infrared spectra of mixture Vanillin with acrylic showed the above bands although these bands were appeared in the same regions of the acrylic, but it can be characterized in the bands at 1450-1600 cm^{-1} regions that are not founds in acrylic polymer, (Figure 4.9).

The infrared spectra of Raspberry additive samples, (Figure 4.10) showed band addition to acrylic bands (MMA bands) at 688cm^{-1} as Figure (4.8) which due to the Raspberry pigment. The spectra of $TiO_2$when added to acrylic give addition band at 841cm^{-1} (Figure 4.11). The same information gets from the spectra Amaranth pigment as Figure (4.12), Curcumin pigment (Figure 4.13) and Vertex™ synthetic stain additive (stain No.240 10%) (Figure 4.15) that gives a band at 668cm^{-1} region.



The other bands of synthetic™ stain (stain No.240 1%, stain No. 210 5%, stain No. 220 10%, Stain No.250 1%) did not appear in the spectra because the spectra of elements appeared between 200 - 400 cm^{-1} and this bands out of the range of machine used as in Figures (4.14, 4.18, 4.16 and 4.19) respectively.

Figure (4.8): Infrared spectrometer chart of the monomer (methyl methacrylate).

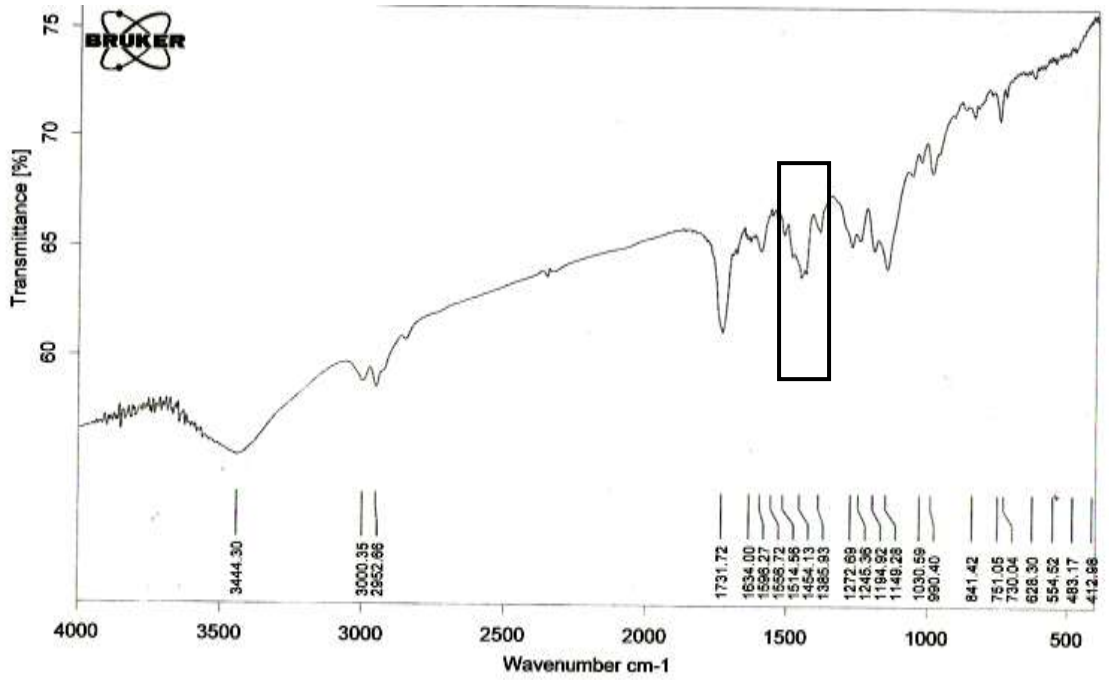


Figure (4.9): Infrared spectrometer chart of the sample prepared with the addition of Vanilla.

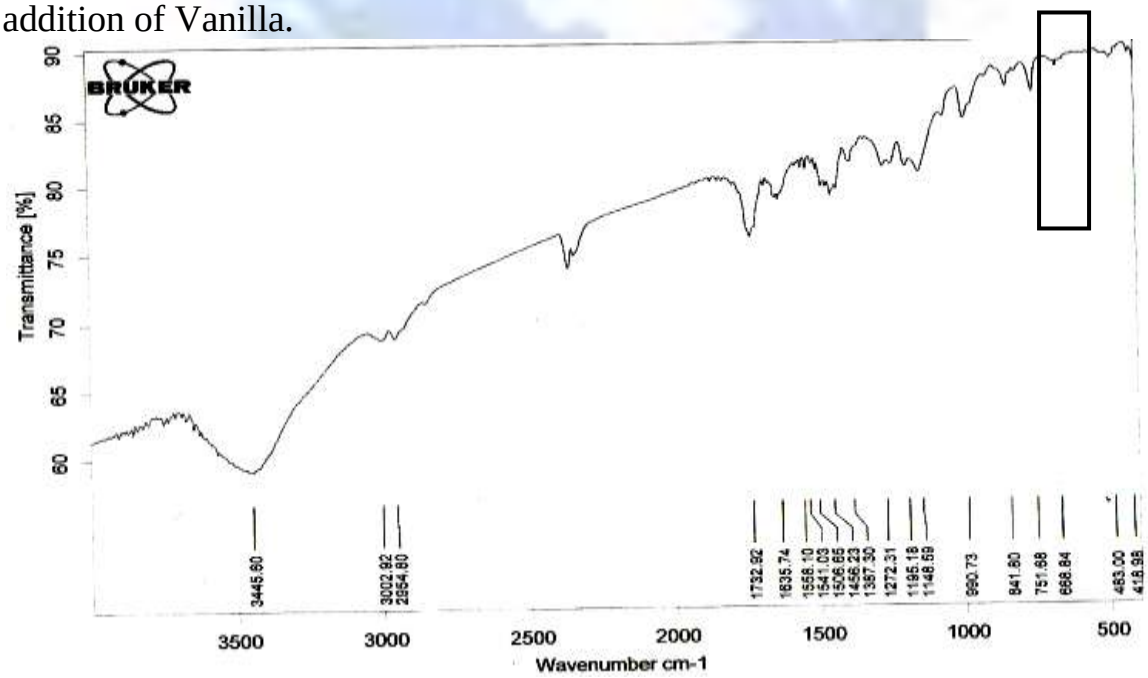


Figure (4.10): Infrared spectrometer chart of the sample prepared with the addition of Raspberry.

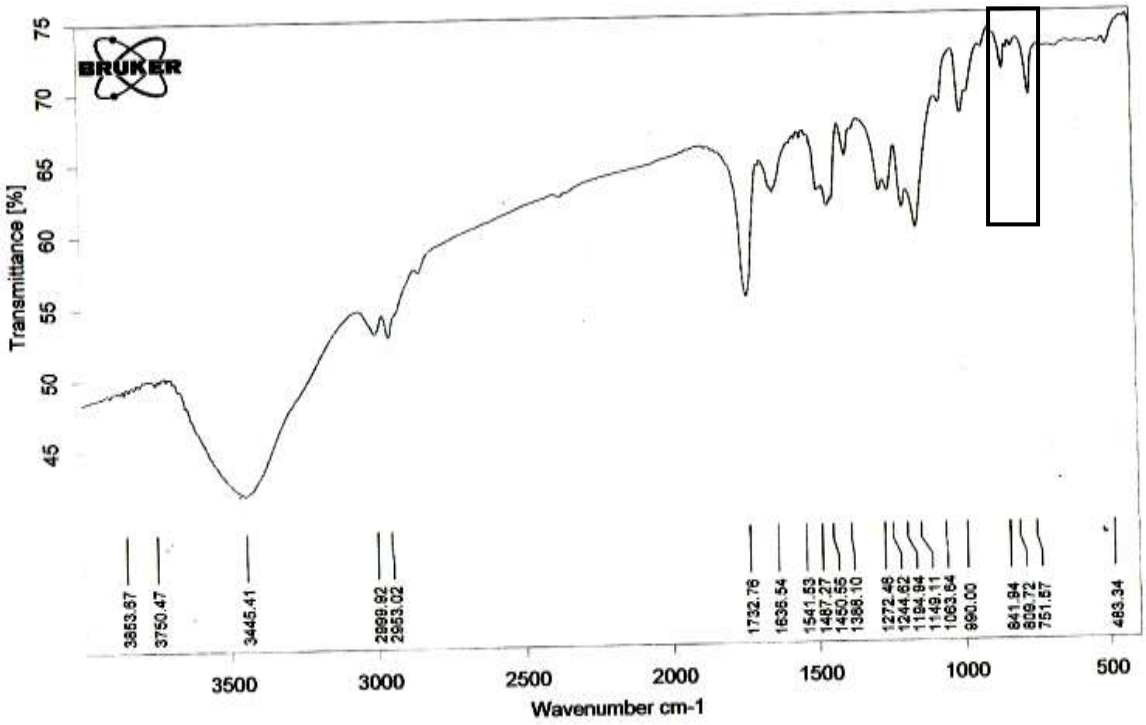


Figure (4.11): Infrared spectrometer chart of the sample prepared with the addition of TiO_2.

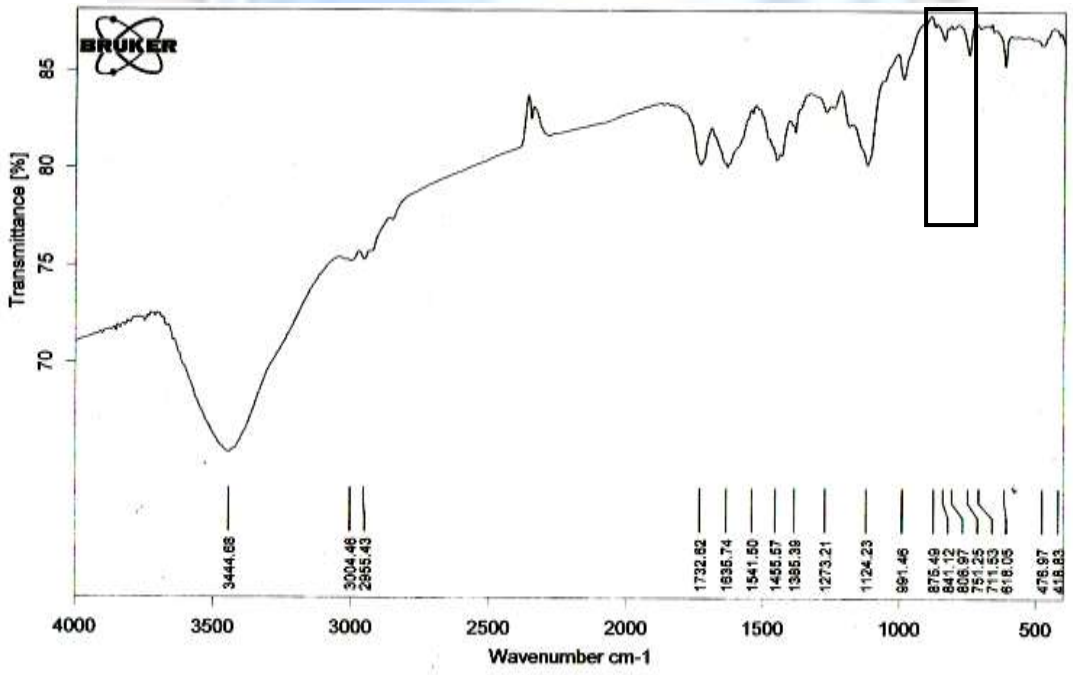


Figure (4.12): Infrared spectrometer chart of the sample prepared with the addition of Amaranth.

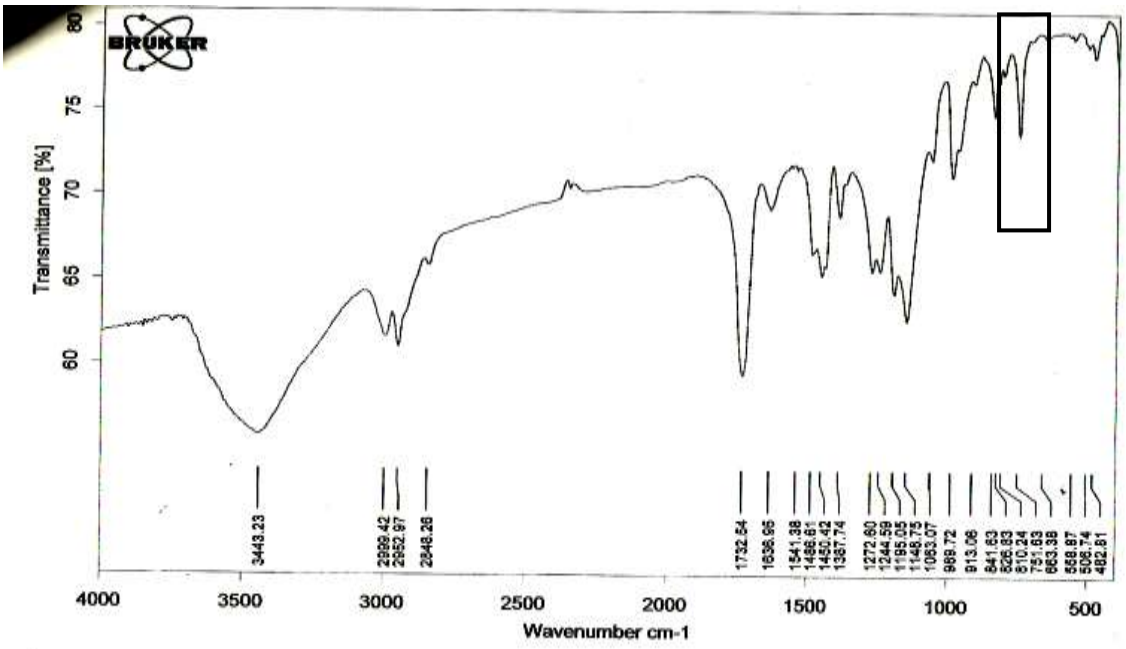


Figure (4.13): Infrared spectrometer chart of the sample prepared with the addition of Curcumin.

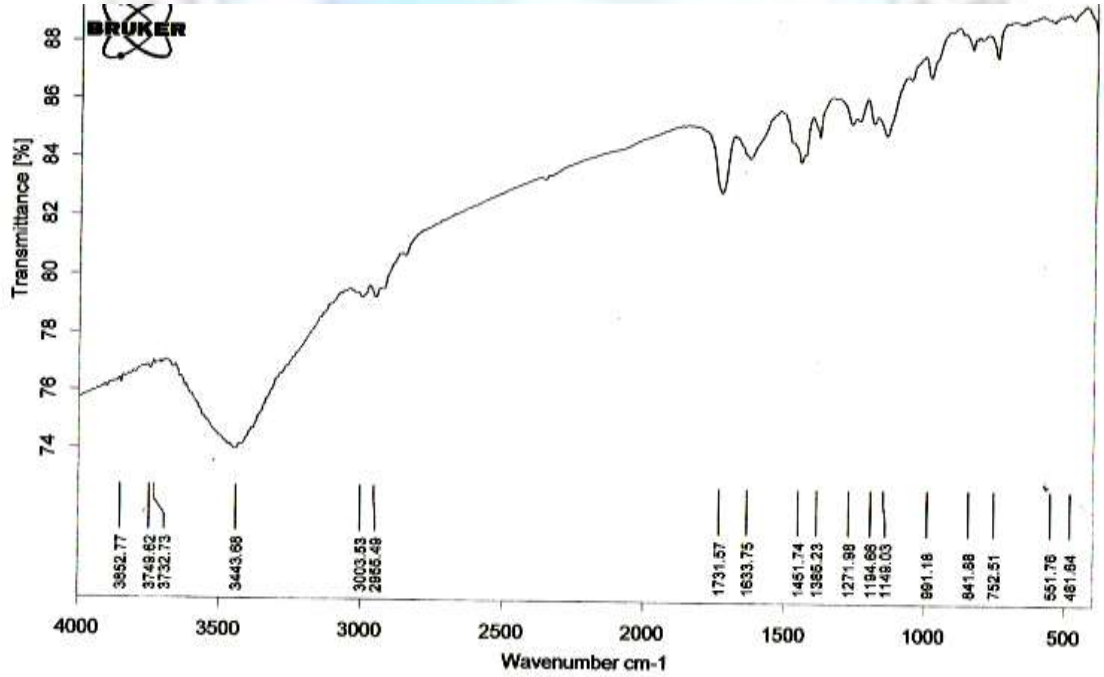


Figure (4.14): Infrared spectrometer chart of the sample prepared with the addition of synthetic™ stain No.240 (1%).

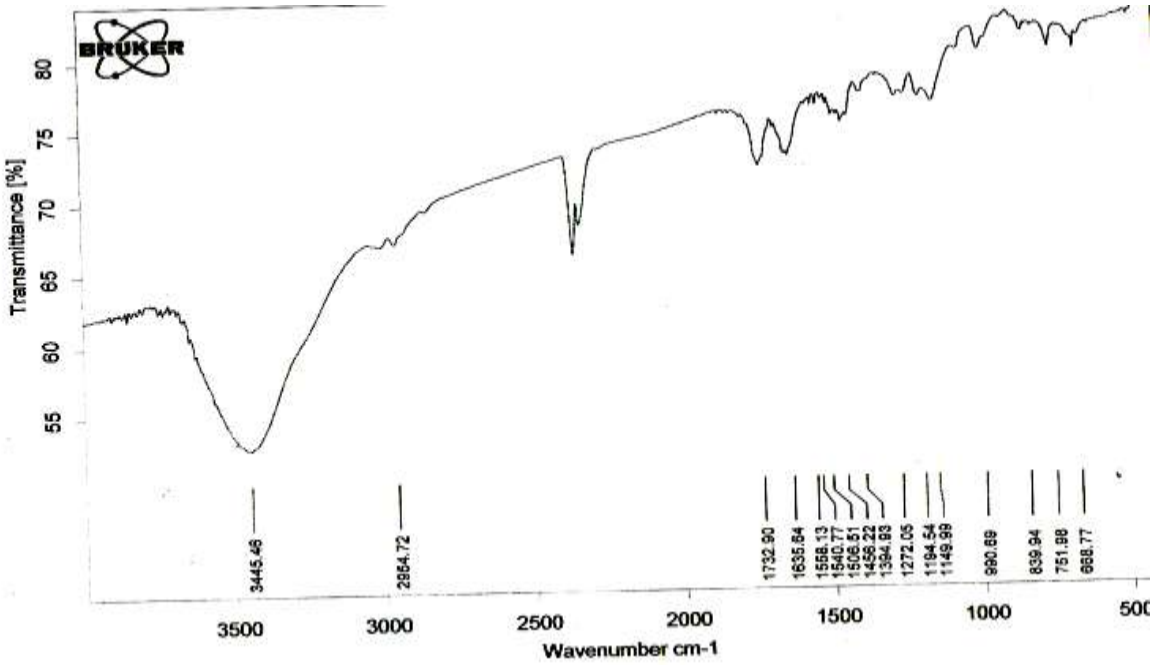


Figure (4.15): Infrared spectrometer chart of the sample prepared with the addition of synthetic™ stain No.240 (10%).

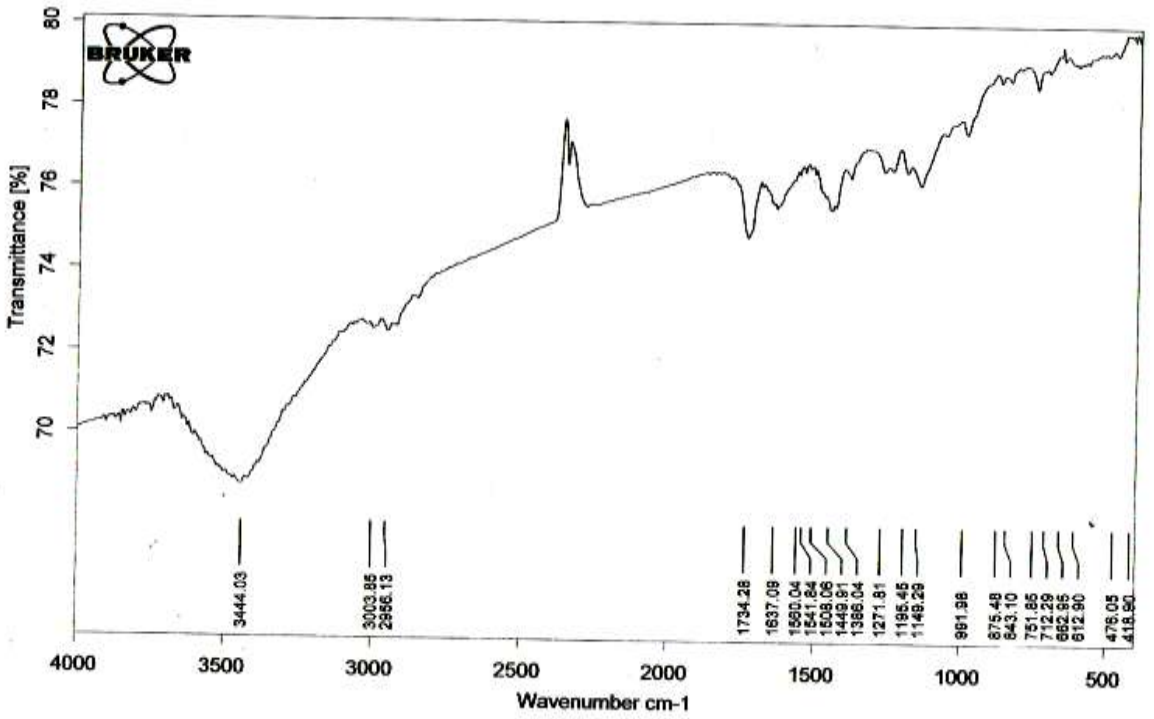


Figure (4.16): Infrared spectrometer chart of the sample prepared with the addition of synthetic™ stain No.220 (10%).

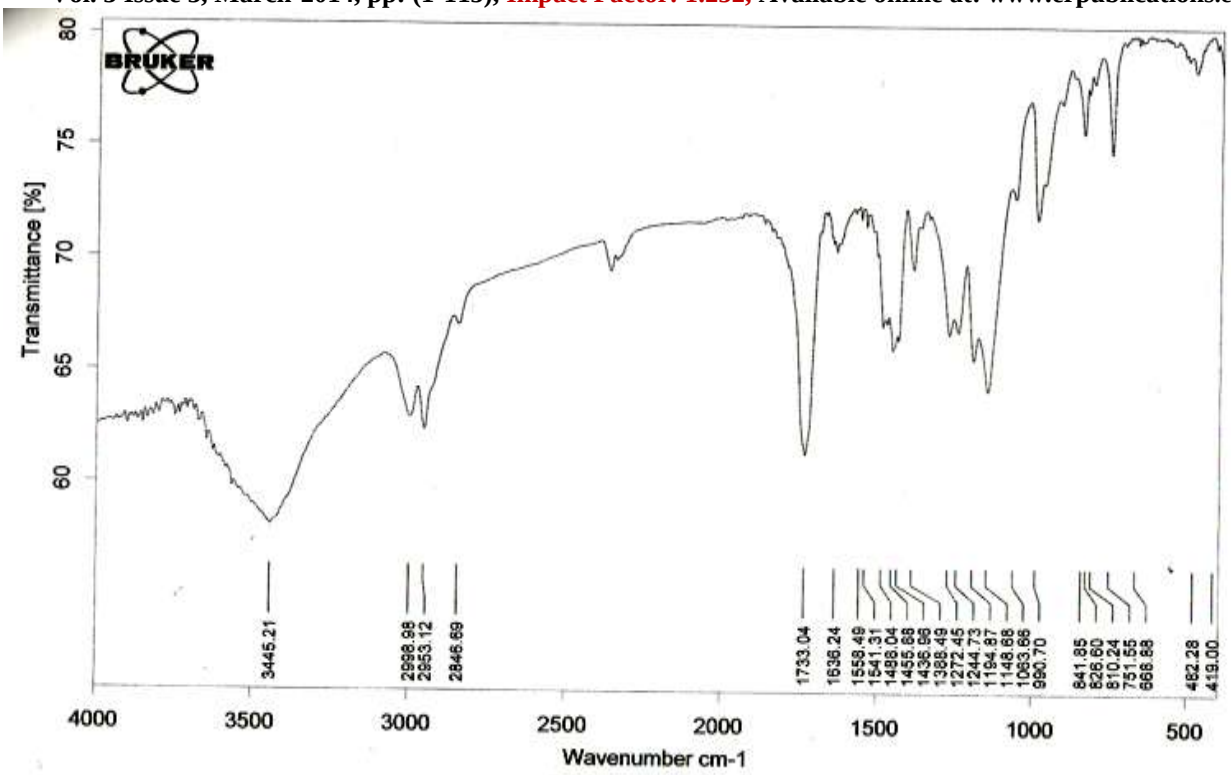


Figure (4.17): Infrared spectrometer chart of the sample prepared with the addition of synthetic™ stain No.220 (5%).

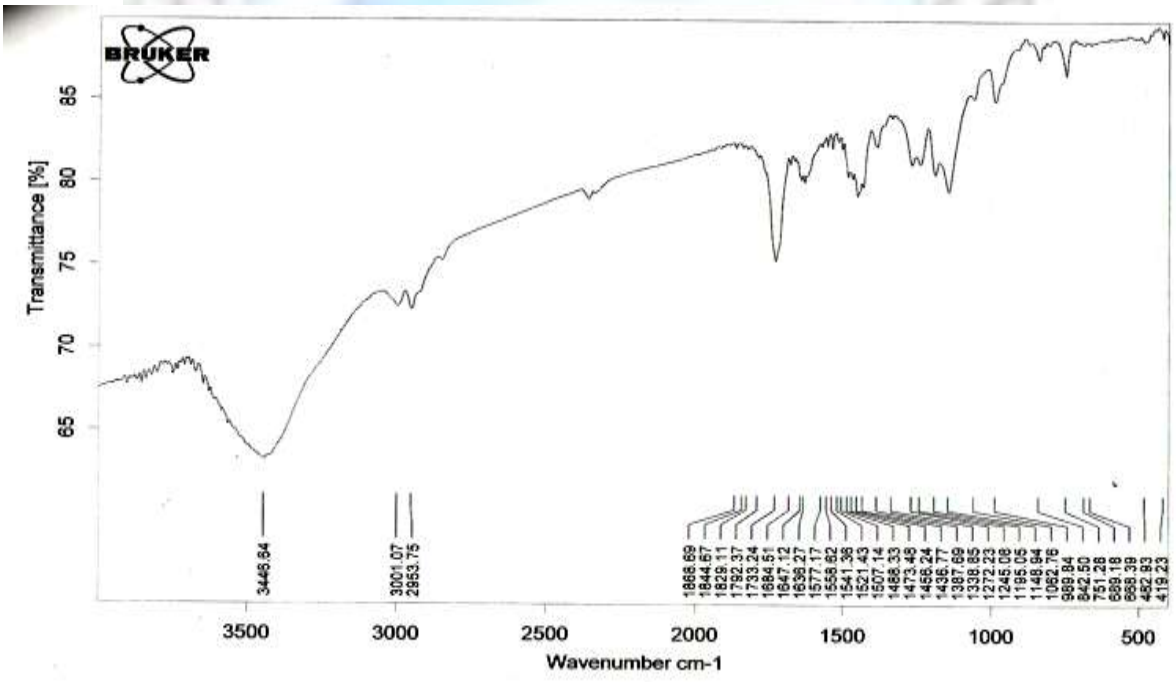


Figure (4.18): Infrared spectrometer chart of the sample prepared with the addition of synthetic™ stain No.210 (5%).

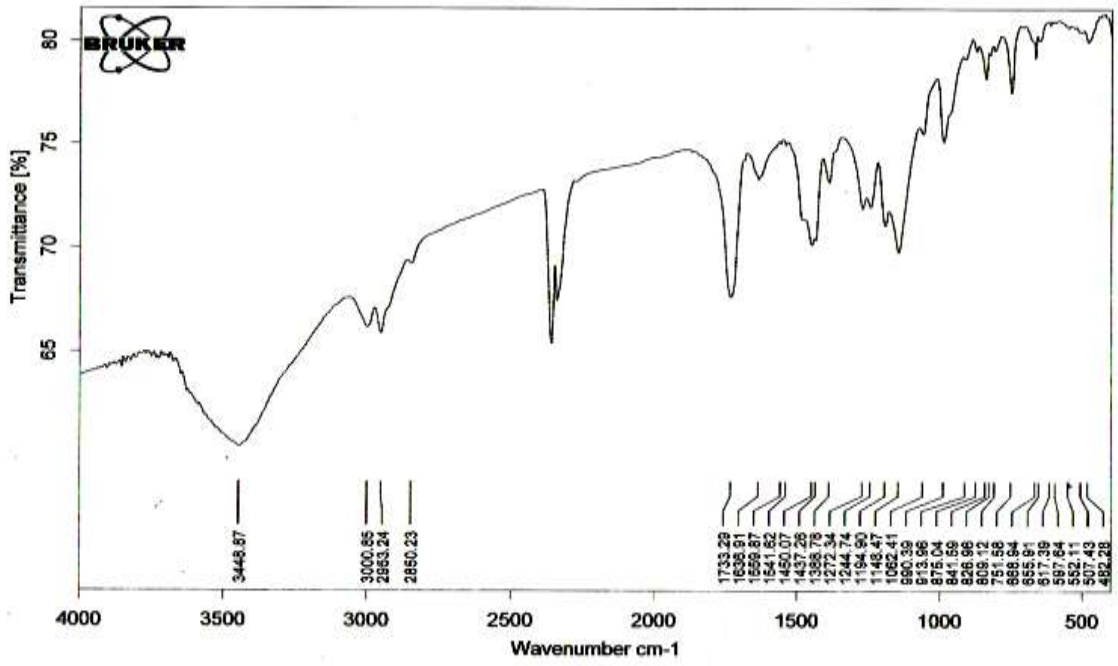


Figure (4.19): Infrared spectrometer chart of the sample prepared with the addition of synthetic™ stain No.250 (1%).

4.8 Microscopic Examination of the Prepared Polymer

Examination of the Vertex™ acrylic polymer with both natural and Vertex™ synthetic additives under light microscope (magnification power X10) showed that the particles size of the additives were smaller than the polymer particles, and the shape of the Vertex™ synthetic stain, Amaranth, TiO_2 pigments were spherical, while Vanilla, Raspberry and Curcumin pigments were irregular in shape as in Figure (4.20).

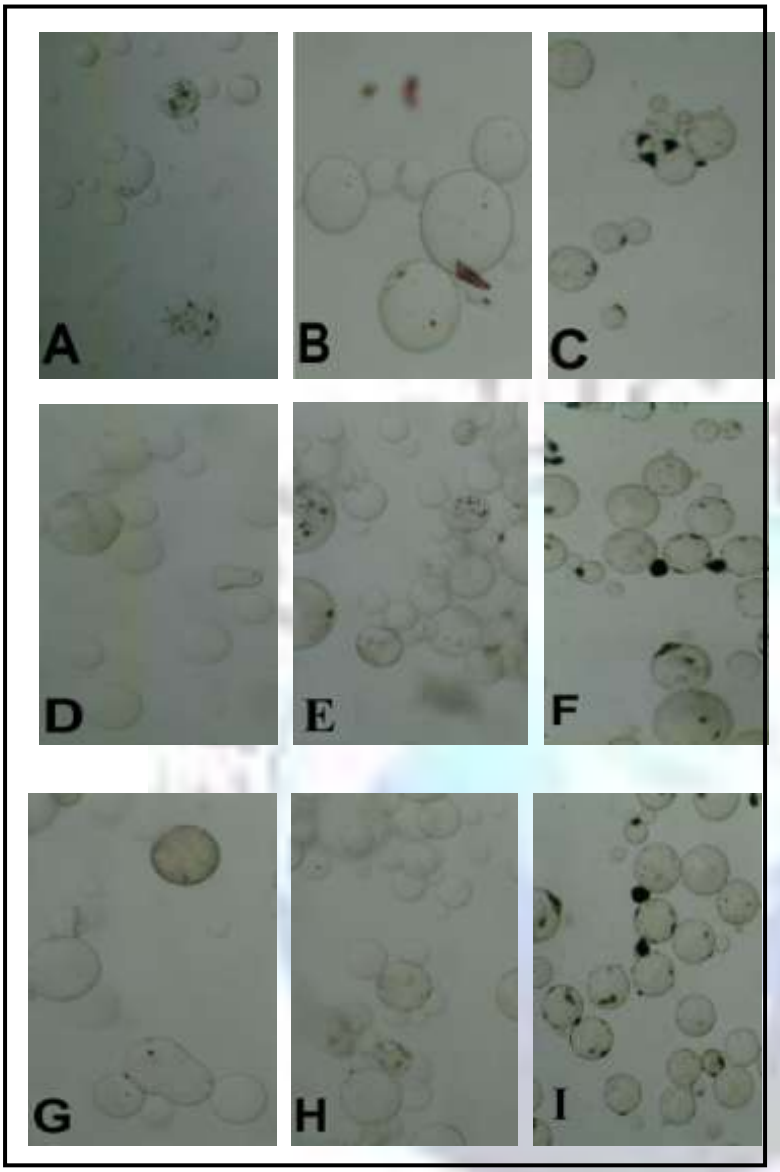


Figure (4.20): Microscopic examination of prepared powder (acrylic powder with additives);

A: Amaranth additive, B: Raspberry additives, C: Titanium dioxide additive, D: Vanilla additives, E: Curcumin additive, F: synthetic stain No. 220 additive, G: synthetic stain No. 210 additive, H: synthetic stain No. 250 additive and, I: synthetic stain No. 240 additive.

CHAPTER FIVE

DISCUSSION

This is a recent study of natural pigments (Amaranth, Raspberry, Titanium dioxide, Vanilla and Curcumin) that used as coloring agent additives material to heat cured acrylic resin denture base material to prepare artificial gingival shade guide instead of that available from synthetic stains, and there are no previous studies to correlate the results of this study with it.

5.1 Measurement of Color Property

Figures (4.1 and, 4.2) represented the colors of the used natural pigments and Vertex™ synthetic stains added to translucent polymer according to (L*a*b*) graph system to denote the real color of each natural pigment and synthetic stain alone without interaction with effect of the pink color of the pink polymer. The 3D graphs showed that all used coloring additives with different concentrations (each natural pigment and synthetic stain added with different concentrations to denote the effect of increasing concentration of the additive and to compare with control sample) were located within the same side that required to obtained the color grade (the obtained values were located in positive side according to (L) axis that mean color tend toward whiteness, in (a) axis the obtained values located in positive side this mean tend to red color, values related to (b) axis located in positive side that mean tend to yellowness) agreement with Baltzer and Kaufmann (2004).

Samples measured with different concentrations of each pigment (Amaranth, Raspberry, TiO_2 and Curcumin) decreased the (L) value within positive side, that means the lightness was decreased. Vanilla increased the

(L) value in the positive side that mean the lightness was also increased. This is confirmed by comparing the control samples (without additives), these results are showed in Figure (4.1) and Table (4.1). All the Vertex™ synthetic stain used (stain No.210, 220, 230, 240, 250) decreased (L) value; that means decreased the sample lightness (Figure 4.2) and (Table 4.1), this agreed with Billmeyer and Saltzman (1981).

Amaranth, Raspberry and Vanilla Samples (each pigment prepared with multiple concentrations to show the effect of increasing concentration in color graph) gave grades of red color and with increased concentration of the additives the value of (a) increased in the positive side, that means the redness was increased. This explained by the red color of the pigments. This was clearly showed in Figure (4.1) and Table (4.1) and confirmed by comparing with the control sample (without additives) that (a) value was close to zero. TiO_2 and Curcumin pigments decreased the redness with increasing concentration of the additives. The value of (a) decreased within the positive side; that means the redness was decreased. All the Vertex™ synthetic stain used (stain No.210, 220, 230, 240, 250) increased (a) values, that means increased sample redness (Figure 4.2) and Table (4.1) agreement with Billmeyer and Saltzman (1981), Wyszecki and Stiles (1982) and Hutchings *et al.* (2002).

Value (b) of the samples with Amaranth, Raspberry, Vanilla, TiO_2 and Curcumin pigments (each pigment added with multiple concentration to illustrate the color changes with increasing concentration) have a degree of yellowness (tend toward yellow color) that all the values of (b) color are positive. When increase concentration of the additives the value of (b) increased in the positive side (Figure 4.1) and Table (4.1) and this confirmed by comparing with (b) value of the control sample (without additives). Amaranth, Raspberry, Vanilla, TiO_2 and Curcumin increased yellowness.

Curcumin pigment gave the highest value of (b) yellowness, this belongs to yellow color. All the Vertex™ synthetic stain used (stain No.210, 220, 230, 240, 250) increased (b) value that mean increased yellowness of samples as in Figure (4.2) and Table (4.1) agreed with Billmeyer and Saltzman (1981) and Bunting (1998).

Dr.- lng. Wolfgang Rauh (2012) a director Business Unit – Dental Devices in VITA company gave information that "can be used Easyshade to compare different samples of resins just be comparing the reported values but that the values will not correspond to values provided by other devices and similar to resins it be possible to use it to compare different gingiva but the values will not be absolute values".

Results in Table (4.4) showed that addition of Amaranth to pink polymer as prepared (G1) gave hue (H) color value same as in the control pink sample (without additives) (G12), but all the other color values (L, a, b, C) was changed, that addition of Amaranth will give hue value (main color) within the grades of the pink color, that is most color required in clinical uses, this agreed with Myers (2002) and Ghodake *et al.* (2011).

Results in Table (4.4) showed the addition of Vanilla in (10%) wt/wt to pink polymer samples (G4) increased the (L) value (lightness) in comparison with lightness of the control sample (pink polymer samples without additives) (G12) (Ishikawa-Nagai *et al.*, 2004), while the other color values (C, H) remained as it. This refers that it can be using Vanilla to increase the lightness value in relation to control.

Results in Table (4.5) showed that gingival color values of the patient (13ma) matched the color of sample with Amaranth additive (0.1%) (G1) that (ΔE=5.01). Adding mixture of Vanilla and Amaranth to pink polymer showed decreasing in the color difference of the mixture to (ΔE=3.3). So, adding Vanilla as a secondary additives would reduce the color difference, this may be due to the effect of Vanilla in increasing the lightness value.

The (ΔE) of the human gingival color values versus samples prepared with Vanilla additives (10%) (G4) was acceptable in vivo and the (ΔE) with synthetic stain No. 240 in (10%) (G7) was also acceptable in vivo as results in Table (4.5) that showed the patient's gingival color values (6fb) matched both Vanilla additive (10%) (G4) and samples with synthetic stain No. 240 (10%) (G7). So, it can substitute the use of Vertex™ synthetic stain No. 240 by the natural Vanilla.

The measured human gingival color values matched both concentration (5%) and (10%) of the synthetic stain No.230, that (ΔE) of (5%) additive concentration was (ΔE=6.017) and (10%) additive concentration was (ΔE=6.120) that both were accepted in vivo and the difference in between was very small, also seen that synthetic stain No. 240 (1%) and (10%) additive concentrations matched with same human gingival color values that (ΔE) of 1% was (ΔE=5.47) and 10% was (ΔE=5.49), and in synthetic stain No. 250 (1%) and (5%) matched with same human gingival color values, that (ΔE) of 1% was (ΔE=4.96) and (5%) was (ΔE=5.52) as shown in Table (4.6). This confirms that increasing this synthetic color additive concentration from (5%) to (10%) in stain No.230, from (1%) to (10%) in stain No. 240, and from (1%) to (5%) in stain No.250 would not cause a noticeable difference in color values, from that it requires more quantity of the synthetic color additive to obtain another grade of the main color and this is a waste of the material and consequence higher the cost, this is agreed with Nia *et al.* (2009).

The color values of human gingival (6fb) matched synthetic stain color No. 220 (1%), No. 230 (1%) and No. 240 (1%) that (ΔE) between everyone of them with the human gingival color was accepted in vivo that showed the little difference between the three colors in (1%) addition (that the three stains in the same kit) as shown in Table (4.7).

Table (4.8) showed that results from the three different concentrations (1%, 5%, 10%) wt/wt prepared for each individual stain of Vertex™ kit stains [contain five colors (stain color No.210, color No.220, color No.230, color No.240, color No.250)] only matched two colors from all the (24) patient's gingival color measured, and matched only one color from colors of the 10 Tabs of the Vertex™ gingival shade guide, while (4) colors matched with samples prepared of Vanilla, Amaranth, mixed Amaranth with Vanilla addition.

This may explained that most of the differences in gingival color were in the lightness value and the Vanilla addition modify the lightness value, this agreed with Bayindir *et al.* (2009).

Color ratings for the maxillary interincisal papillae were located more towards the yellow hue (Heydecke *et al.*, 2005). For this reason, Curcumin was used as coloring additive in this study, but no results appears that it's matched with the measured patients gingival color. This may due to a limited number of measured patients or to the limited concentrations that were tested of Curcumin in this study.

Color property were measured for all the prepared groups (N=749), while only twelve different groups as mentioned in section (3.4.1) and (Figure 3.12) (G1; Amaranth 0.1%, G2; Raspberry 0.01%, G3; TiO_2 1%, G4; Vanilla 10%, G5; Curcumin 1%, G6; stain No.240 1%, G7; stain No.240 10%, G8; stain No.220 5%, G9; stain No.220 10%, G10; stain No.210 5%, G11; stain No.250 1%, G12; pink control) were selected among all other prepared groups

to continue the other property tests (indention hardness, volumetric changes, calculate of water sorption and solubility, measurement of residual monomer concentration, FTIR and microscopic examination tests), because there were large number of prepared groups that cannot tests all of them. For this reason, only twelve groups chosen, some were nearly matched by hue value (H) (the main color) between natural, synthetic coloring additives, Vertex™ gingival shade guide as in Table (4.9). Others were chosen to include all other additive types (that measured in color property) into other tests measured in this study. At least, one or more groups from each additive type prepared in this study (from the used five natural pigments, from kit of Vertex™ synthetic stain that contain five colors) underwent all mechanical, chemical and physical property tests in this study.

5.2 Indentation Hardness Test

In this study, the Rockwell hardness test was used. The results in Table (4.10) showed that there was decrease in indentation hardness of samples with synthetic stain No.240 (10%) (G7), samples with synthetic stain No.220 (10%) (G8), samples with synthetic stain No.210 (5%) (G10) and samples with Curcumin in 1% (G5) in comparison with control sample (G12) as shown in Figures (4.3). This could be explained due to the high value water sorption value, this agreed with Cury *et al*. (2001) and Braun *et al*. (2003). Issac (1992) found that the mean value of Rockwell hardness number is in inverse relation to the percentage water content of hardness test samples.

Samples with natural pigments TiO_2 in (1%) increased the indentation hardness, this agreed with (Al- Anie *et al.,* 2010) explained that TiO_2 have sufficient surface area help to make good adhesive with polymers that increase hardness, also change in hardness could be explained by water sorption

phenomenon that seen from the results in Figure (4.5) TiO_2 was the lowest value water sorption of all the tested group and the highest hardness value as in Figure (4.3). With agreement with Garcia *et al.* (2004), Azevido *et al.* (2005) and Campana *et al.* (2005) those denoted that water as small molecules, may act as a plasticizer following diffusion into the polymer, thus relaxing the polymer chains and subsequently lowering the hardness of the acrylic resin denture base.

5.3 Volumetric Changes Test

Figure (4.4) and Tables (4.11 and 4.13) showed there were no significant changes in volume mean after two days immersion in non ionized distilled water, prepared samples with natural pigment additive (Amaranth, Raspberry, Titanium dioxide, Vanilla, Curcumin) showed more dimensional accuracy than the control sample. This may due to the particles of the pigment that fills the gaps between polymer chains and reduce the amount of water absorption and subsequently reduce the volumetric changes, this agreed with McCabe and Walls (2008).

Samples prepared with synthetic stain No.240 10% (G7), No.220 10% (G8), No.250 1% (G11) also showed more volume accuracy (0.0%) than control sample (G12) that showed (0.05%) volume changes, while samples with stain No.220 5% (G9) show increase in volume (0.03%), samples with synthetic stain 210 5% (G10) showed decrease in volume (0.08%) (Table 4.11). This may due to the polymerization process or the amount of residual monomer, this agreed with Abdul Razzak (2010) and Hatim *et al.* (2010).

All prepared samples in this study that increased or decreased in volume were not significant and within the limit of polymerization shrinkage and distortion that (8%) approximately supported by Consani *et al.* (2002).

5.4 Calculate of Water Sorption

Figure (4.3) and Table (4.14) showed the results of this study that the mean value of water sorption for control samples (G12) was (20.83 µg/mm^3). The maximum mean value of water sorption of the samples prepared with natural pigment additive was for samples with Curcumin (1%) additive (G5) that was (26.38 µg/mm^3), and the maximum mean value for the synthetic stains was (25.69 µg/mm^3) for the stain No. 210 (5%)(G10). The lowest mean values of samples with natural additives were for samples with Titanium oxide additive (1%) (G3) was (18.05µg/mm^3), while samples with synthetic stain No.220 (10%) (G8) had the lowest mean value between samples with synthetic additives (19.44µg/mm^3). All the water sorption values for natural and synthetic additives were accepted with ADA specification No.12 (2002) that is (32 µg/mm^3 as maximum).

The amount of water sorption may be related to the presence of additives particles that irregular in shape that incorporate gaps between polymer chains this agreed with Arora *et al.* (2011) that explained lower sorption value than the control by incorporation of additives to decrease the potential sites of water exchange to occur.

5.5 Calculate of Water Solubility

Figure (4.4) and Table (4.15) showed that the water solubility values of control samples and all prepared samples with additives. Samples with Raspberry additive was significant decreased in water solubility. Reducing of solubility may due to decrease the potential sites of water exchange to occur (Arora *et al.*, 2011). The amount of solubility of Curcumin (G5) and Amaranth additive (G1) (0.69µg/mm^3) that higher than the control (G12). This may be due to the small amount of residual monomer released or it may

indicate to the pigment additives that are unable to incorporate and leached out, this supported by Sofou *et al.* (2005).

Water solubility values of the control group (G12) and all the prepared samples with additives were accepted with ADA specification No.12 (2002) that is (1.6 µg/mm^3 as maximum).

5.6 Measurement of Residual Monomer Concentration

The result of daily residual monomer release immersed in non ionized distilled water for all groups (samples without additive, samples with synthetic stain, samples with natural pigments) over continuous seven days showed different level of residual monomer release, then decreased in the remaining monomer release until reach zero (day by day). Group with Curcumin additive (1%) (G5) showed no residual monomer release, groups with synthetic stain additive No.210 (5%) (G10) and synthetic stain additive No.250 (1%) (G11) showed complete elimination at first day, groups with Amaranth (0.1%) additive(G1), Raspberry (0.01%) additive (G2) and Vanilla (10%) additive (G4) showed complete elimination at second day, samples with synthetic stain additive No.220 (5%) (G9) showed complete elimination at third day, control group (G12) and groups with synthetic stain additive No.240(1%) (G6), No.240 (10%) (G7), synthetic stain additive No.220 (10%) (G8) showed complete elimination at fourth day, (G3) group with Titanium dioxide additive showed complete elimination at sixth day, as in Figure (4.7).

Different mechanisms might help to explain this reduction; it has been observed that the concentration of the residual monomer in the polymerized resin can be diminished by diffusion into water and by continuous polymerization promoted by the active radicals found in the polymer chains, agreed with Bartoloni *et al.* (2000) and Abdul-Razzak (2010).

Abdul Razzak (2010) stated that curing the resin by following the manufacturer instructions curing cycle of the Vertex ™ regular acrylic resin provided the optimal properties regarding the highest conversion and lowest amount of released residual monomer.

According to ADA specification No.12, (2002) stated that maximum percentage of residual monomer contents (2.2%) per weight, all of the tested prepared groups were within this limit, that maximum value for the natural pigment additive was (1.71 %), and for the synthetic stain additive was (1.56 %).

5.7 FTIR Test

Figure (4.8) infrared spectrometer chart of the monomer (methyl methacrylate). 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}) (Parikh, 1974; BAHL and Bahl, 2010).

Vanillin spectrum was examined between (1500 cm^{-1}) and (4000 cm^{-1}). There are six major peaks in this region. O−H stretch due to the OH group on the ring−H ring hydrogen stretch,C−H3 C−H stretch in the methoxy (O-CH3) group, HC=O C=O stretch in the aldehyde group, C=C two peaks due to the ring C=C stretch (Parikh, 1974; BAHL and Bahl, 2010). The infrared spectra of mixture Vanillin with acrylic showed the above bands although these bands were appeared in the same regions of the acrylic because they contain same groups in this composition, but we can characterize in the bands at (1450-1600 cm^{-1}) regions in Figure (4.9) which due to aromatic ring which was not in acrylic polymer. The infrared spectra of Raspberry additive showed bands addition to acrylic bands at 688cm^{-1} (Figure 4.8) which due to the Raspberry pigment, the spectra of Titanium oxide when added to acrylic give addition

band at (841cm^{-1}) (Figure 4.11). The same information gets from the spectra synthetic stain additive No. 240 10% (G7) that gives a band at 668cm^{-1} region (Figure 4.15), and this agreed with Anehosur *et al.* (2012) who stated FTIR results showed significant and adequate bonding between TiO_2 and PMMA.

The other bands of the other synthetic stains not appeared in the spectra because the spectra of elements appeared between (200-400 cm^{-1}) and these bands are out of the range of machine used, this agreed with Sarbu *et al.* (2004).

5.8 Microscopic Examination

Figure (4.20) showed that the particle size of the additives was smaller than the polymer particles. This result could be explained the fact that the smaller particle size provides the positive advantage to the topography of the denture base plastic, this is in agreement with Kazanji and Al-Kazzaz (2002).

The shape of the Vertex™ synthetic stain, Amaranth, Titanium dioxide pigments was spherical, while Vanilla, Raspberry and Curcumin pigments were irregular in shape. Titanium dioxide particles were very small in size and regular spherical in shape in compare to polymer particles, the prepared samples with this additive showed higher hardness in comparing with control samples, this was due to the more compact dense particles in polymerized sample, this is supported by Al- Anie *et al.* (2010).

Raspberry, Vanilla and Curcumin powder were irregular in shape, this may explain the difference in volumetric changes of the prepared samples in comparing with control sample that create more gaps between polymer chains. There was no applicable work of these natural coloring additives to acrylic resin denture base material to compare the results.

CHAPTER SIX

CONCLUSIONS AND SUGGESTIONS

6.1 Conclusions:

The current study concluded the following:

1. Prepared three shades of artificial gingival shade guide as results of color property test that approved the using of natural pigments (Vanilla 10% wt/wt or Amaranth 0.1% wt/wt as single color additives and mixture of Vanilla with Amaranth in different concentration) instead of the synthetic Vertex™ acrylic stains No.(210, 220, 230, 240) in different concentrations is clinically acceptable compared in relation to patients' attached gingiva and Vertex™ gingival shade guide.
2. Two consequence concentration of the synthetic color (1%, 5%, 10% wt/wt) produce; very near color values that in vivo match the same patient, so staining effect of Vertex™ synthetic acrylic stains to produce the next grade of the color requires higher quantity than in relation to the natural pigments.
3. All the results of mechanical and physical properties of all the tested groups were within the range of acceptance according to ADA specification No.12, (2002).
4. The results of infrared spectra indicated that the product from the natural pigments, and synthetic stain additives mixed with acrylic resin that used in this study is a homogenous compound between the additive and acrylic without any effects for the property of the additive and acrylic respectively.

6.2 Suggestions:

The current study suggested the following:

1. The system needs further laboratory and clinical testing, and requires a large number testing for different concentration of additives and large number of subjects to be surveyed. Take into consideration, the limited number of the patients surveyed, there are a number of synthetic stains in kit not matched like in the results of stain No.250 (grade of brown color), that this is mainly specified for the dark people
2. Biocompatibility test is needed to discuss the effect of the studied coloring agents. Although, all the natural pigments used acceptable by British pharmacopoeia.

3. Evaluate the color properties of the prepared samples over extended period of time to study the color shelf life of these coloring agents additives (after 6 months, one year).
4. Study the color matching between natural additive versus synthetic additive materials to heat cured acrylic resin denture base, that cannot discussed in this study because it's huge result, more studies needed to focus in this point.
5. Study mechanical and physical properties of the samples with mixed two natural additive that not tested.
6. Study another mechanical tests like tensile and compressive strength tests.
7. Adding the natural pigments to the monomer instead of additions to the polymer and study the color results.
8. Incorporate more natural pigments and add mixture of them to the acrylic to obtain more colors and grades such as iron oxide, red cherry pigments.

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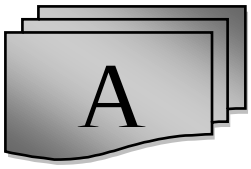

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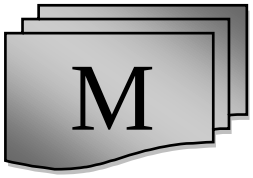

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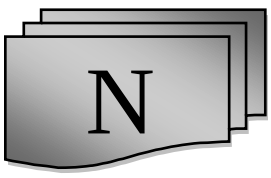

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