Journal Homepage: - www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/18621
DOI URL: http://dx.doi.org/10.21474/IJAR01/18621

RESEARCH ARTICLE

SOLUBILITY AND BIOMINERALIZATION ABILITY OF PMMA BONE CEMENT NANOCOMPOSITE FILLED WITH 40% NANOHYDROXYAPATITE FOR USE AS ROOT-END FILLING MATERIAL

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Manuscript Info

Manuscript History
Received: 28 February 2024
Final Accepted: 31 March 2024
Published: April 2024

Key words:-
Polymethyl-Methacrylate Bone Cement, Mineraltrioxide Aggregate, Nanohydroxyapatite, Biomineralization Ability, Solubility, Root End Filling

Abstract

Aim: This study was conducted to compare and evaluate the solubility and biomineralization ability of polymethyl-methacylate bone cement filled with prepared calcium phosphate based nano-fillers versus mineral trioxide aggregate; when used as root end filling material.

Materials and Methods: A total of forty two samples were prepared and classified into three main groups**(n=14)**according to the material type: Group I: white mineral trioxide aggregate, Group II: Polymethyl-methacrylate bone cement and Group III: Polymethyl-methacrylate bone cement loaded with 40% of prepared calcium phosphate based nanofillers**.** Each material type was mixed and packed into molds. Solubility testing was done following the ISO specification #4049 (2009). For the biomineralization ability testing, each specimen was immersed in 150 ml of simulated body fluid for 21 days. After that, the surface of the cements was analyzed by scanning electron microscope and energy dispersive X-ray analysis with computer-controlled software for elemental analysis.

Results: Energy dispersive X-ray analysis results revealed the absence of both calcium and phosphorus elements in polymethyl-methacrylate bone cement samples. On the other hand, both calcium and phosphorus elements were present in the other two tested materials samples. Statistical analysis revealed a statistically significant difference between the tested groups ($p<0.05$). Regarding solubility, MTA (GroupI) was significantly the lowest; having mean value of ($0.010 g/mm^3$), while Bone cement with nanofiller (group III) was significantly the highest; having mean value ($1.009 g/mm^3$).

Conclusion: Within the limitations of the present study, it can be concluded that addition of nanohydroxyapatite to polymethyle-methacrylate bone cement enhanced its biomineralization ability to be capable of apatite formation and on the other hand, it increased its solubility.

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Introduction:-

Non-surgical endodontic treatment is the first line of treatment to avoid surgery. However, surgery is indicated in cases with persistent periradicular pathosis.

As a root-end filling material, mineral trioxide aggregate (MTA) fulfills the requirements of being biocompatible, with biomineralization ability, non-toxic to periradicular tissues and non-resorbable in tissue fluid. However, due to the known drawbacks of MTA such as a long setting time, high cost, and potential of discoloration; the need for innovative root-end filling materials is required.

Calcium phosphate-based compounds as hydroxyapatite, on the other hand, proved to have excellent biocompatibility and interaction with surrounding hard tissue. Nano-sized hydroxyapatite also proved to improve osteogenic differentiation, bone growth and osteointegration compared to that of their micro-scale. However, due to their brittleness, and low strength properties; their widespread use is limited.

Polymethylmethacrylate bone cement is widely used in orthopedic surgery and is commercially available as a two-phase self-curing system. When the powder polymer and liquid monomer phases are mixed, injectable cement is produced. The polymerized cement characterized by high mechanical strength properties, offering structural and mechanical support to a wide range of reparative surgeries together with immediate cure, and ease of handling. The lack of bioactivity of PMMA bone cement is the main drawback of the cement where no chemical or biological bonding will form at the interface with host bone. To overcome these draw backs, loading the matrix of PMMA with different types of fillers has been considered. Addition of 30 wt% hydroxyapatite (HA, Ca10(PO4)6(OH)2) to PMMA matrix was studied by Moursi et al and found to increase the interfacial shear strength after six weeks of implantation. Such finding is in agreement with Moursi et al. who found improvement in osteoblastic response through the addition of HA in a PMMA matrix when compared to unfilled PMMA. Examples of particles that have been tried as fillers to PMMA matrix to improve its properties include; calcium carbonate nanoparticles , silica nanoparticles collagen , and core-shell nanoparticles.

Therefore, this study aimed to modify the composition of the commercial PMMA bone cement through the addition of 40% of a prepared nano hydroxy apatite in an attempt to produce a bioactive cement for use as root end filling material. The solubility and biomineralization ability of the prepared composite were evaluated and compared to that of MTA root end filling material.

Materials and Methods:-

Materials used during this study include; Polymethyl-methacrylate bone cement; (Eurofix Ro) (Synimed, SynergieIngenierieMedicale S.A.R.L, Chamberet-France) and White mineral trioxide aggregate; (Angelus, Londrina, PR, Brasil)

Preparation and characterization of hydroxyapatite nanoparticles:

Hydroxyapatite nanoparticles were prepared using the wet chemical method; where calcium nitrate, di-ammonium hydrogen phosphate, and ammonium hydroxide were used as the starting materials(12). The stoichiometry of the calcium nitrate and di-ammonium phosphate solutions was adjusted to get the (Ca/P) molar ratio close to 1.67(8, 15).The prepared powder was characterized using Fourier transform infrared spectrometer (FTIR), X-ray diffraction (XRD), Scanning electron microscope (SEM)**,** energy dispersive X-ray (EDX) analysis and Transmission electron microscope (TEM)[17].

Samples'grouping and sample size calculation:

A total of forty two samples were prepared, where sample size was calculated depending on a previous study (17) as a reference. According to this study, the minimally accepted sample size was 7 per group, when the response within each subject group was normally distributed with standard deviation 4, the estimated mean difference was 6.5, when the power was 80 % & type I error probability was 0.05.

A total of 42 samples were classified into three main groups **(n=14)** according to the material type:
Group I: White mineral trioxide aggregate;
Group II: Polymethyl-methacrylate bone cement and

Group III: Polymethyl-methacrylate bone cement loaded with 40% of the prepared calcium phosphate based nanofillers**.**

Preparation of Polymethyl-methacrylate /hydroxyapatitecomposite samples:
40 wt % of the prepared hydroxyapatite nanofiller powder was added to the PMMA bone cement powder then were mixed manually using a dry clean stainless-steel spatula in a glass cup. For the preparation of the PMMA bone cement and the prepared composite samples the powder was manually mixed according to the manufacturer's instructions at polymer/monomer ratio of 2:1 using metallic spatula and glass cup. The mix was packed in the prepared molds at the dough stage when the cement separated cleanly from the gloved finger on probing[18].

Preparation of white MTA samples:
The white MTA powder was weighed and mixed according to manufacturer's instructions at a water powder ratio of 1:1. Mixing was done at room temperature using stainless steel spatula and glass slab. The cement was packed using a spatula and then allowed to set in the prepared moulds.

To produce 95 % humidity; wet gauze was placed on the upper and lower surfaces of the cement then were placed in the incubator at 37^{o}C up to 50% longer than the setting time listed by the manufacturer.

Solubility testing:
For the three tested groups, solubility testing was done following the ISO specification #4049 (2009) [11].A total of 21 samples were prepared for solubility testing; n=7 for each tested group. Disc shaped Teflon split molds 15±1mm in diameter and 1.5mm in thickness were fabricated for samples preparation[11]. Each material was mixed and packed into the prepared molds with a nylon thread 5 cm in length embedded into the cement during packing the material into the mold. After complete polymerization and setting, the specimens were removed from the Teflon split molds[11].

The thickness and diameter of each specimen were determined with a digital caliper and the volume (V) was calculated in mm^3 [11].

The specimens were then weighed on a sensitive analytical balance (ADAM, UK) to a precision of 0.0001g; and their weights were recorded as **m.**

Each specimen was then immersed in plastic test tube filled with 20 ml distilled water; and placed in an incubator at 37±1ºC for 24 hours[11].

The specimens were then removed, washed with distilled water, blotted with tissue paper to remove visible moisture and were transferred to a desiccator having anhydrous self-indicating silica gel and left for 48 hours [11].

The specimens were removed from the desiccator and weighed on the analytical balance and this procedure was repeated until a constant mass $\mathbf{m_1}$ was obtained [11].

The **water solubility (Wsol) in g/mm^3** was calculated using the following equation [11]:
$\mathbf{Wsol = (m - m_1)/v}$
where;

- **m=** the mass of the specimen in g before immersion in distilled water.
- $\mathbf{m_1}$ = the reconditioned mass of the specimen in g after being introduced into the silica gel desiccator.
- **v =** the volume of the specimen in mm^3 ; where;

$\mathbf{v = \pi r^2 h;}$

- π = 3.14,
- **r** is the radius of the specimen which is equal to half the diameter of the specimen and
- **h** = the thickness of the specimen.

Biomineralization ability testing:
For biomineralization ability testing, a total of twenty-one samples were prepared using cylindrical Teflon molds 19 mm in diameter and 5 mm in thickness and classified into three groups**(n=7)**according to the material type. [3]. After

setting of MTA and polymerization of the polymer cement samples ,each sample was immersed in 150 ml of simulated body fluid (SBF) for 21 days at 37° C[2].

The SBF was prepared according to the procedure described by Kokubo[2,7].NaCl, NaHCO3, KCl, K2HPO4. 3H2O, MgCl2 . 6H2O, CaCl2 . 2H2O, Na2SO4 and tris-hydroxymethylaminomethane (CH2OH)3CNH2 were dissolved into deionized water and buffered to pH 7.25 at 36.5◦C with hydrochloric acid. The prepared SBF has ion concentration nearly equal to that of human blood plasma[16].

The samples were then mounted on metallic stubs, gold-sputtered and the surface of the cements was analyzed by scanning electron microscope (Tescan Vega 3**,**TescanOrsay Holding, Brno-Kohoutovice, Czech Republic**)**at 500X magnificationfor high resolution imaging**,** conne cted to a secondary electron detector for energy dispersive X-ray analysis (EDX; Bruker, Germany) with computer-controlled software for elemental analysis. Each specimen was observed with an area of 100 µm, magnification 500X, at a distance of 10 mm, resolution of 3 nm and accelerating voltage 30 kV.[3, 4, 9, 12]

Elemental X-ray microanalysis (EDX) provided micro-chemical spectra, element mapping, and semi-quantitative compositional tables (weight % and atomic % of the elemental composition). After collecting the EDX spectra, automatic identification of elements as well as element quantification in both weight % and atomic % was carried out.SEM images and corresponding EDX spectra were specifically observed for apatite formation and identification and quantification of elements. The Ca/P ratio was calculated from the atomic data obtained[16,4].

$$\text{C/P atomic \%} = \frac{\text{calcium mean atomic \%}}{\text{phosphorus mean atomic \%}}$$

Results:-

Characterization resultsof the prepared hydroxyapatite nanoparticles:

Fourier Transform InfraRed (FTIR) spectroscopy analysis results:

The FT-IR spectrum of the hydroxyapatite (HAP) produced by wet method is shown in Figure (1).FT-IR spectrum shows all the characteristic bands for Hydroxyapatite. The asymmetric stretching (v3) and bending (v4) modes of PO_4^{-3} ion were detected at around 1100, and 600 and 586cm^{-1}, respectively. The symmetrical stretching modes (v1 and v2) of PO_4^{-3} ion were also found at around 920.4 and 410 cm^{-1},respectively, while the stretching mode of the OH^- were detected at around 3480 and 1600 cm^{-1},respectively. The stretching vibrations ascribed to CO_3^{2-} at around 1400cm^{-1}were also present.

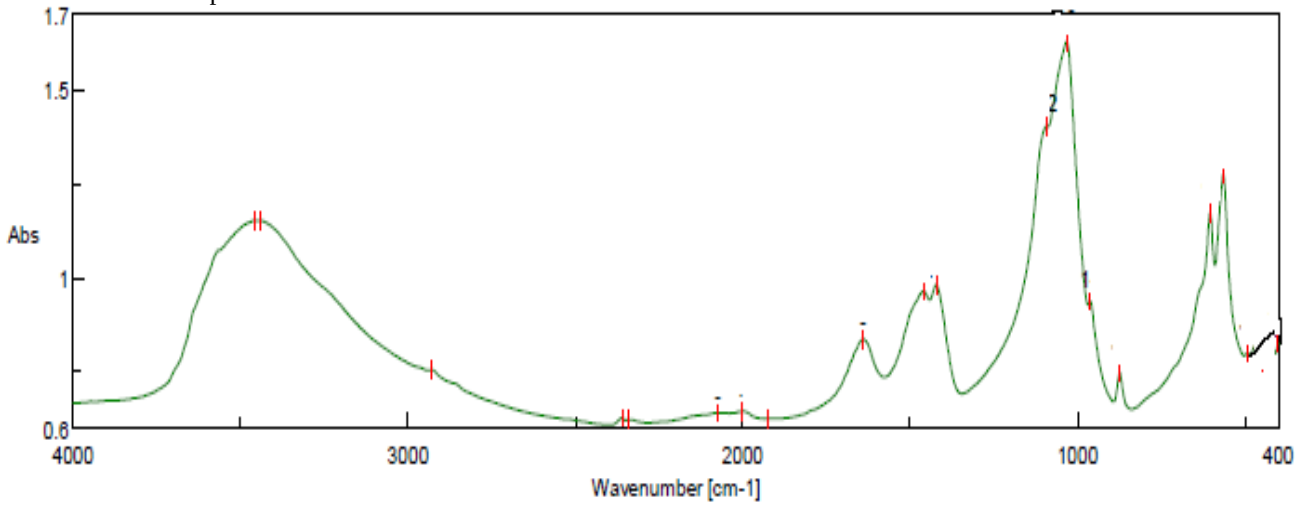

Figure (1):- FTIR analysis of the prepared hydroxyapatite nanoparticles.

X-Ray diffraction (XRD) analysis results:

XRD analysis pattern of the preparedhydroxyapatite nanoparticles is shown in Figure (2). The characteristic peaks of hydroxyapatite werefound and most of the peaks fit well with the hexagonal hydroxyapatite phase.The grain size

D of hydroxyapatite was calculated.

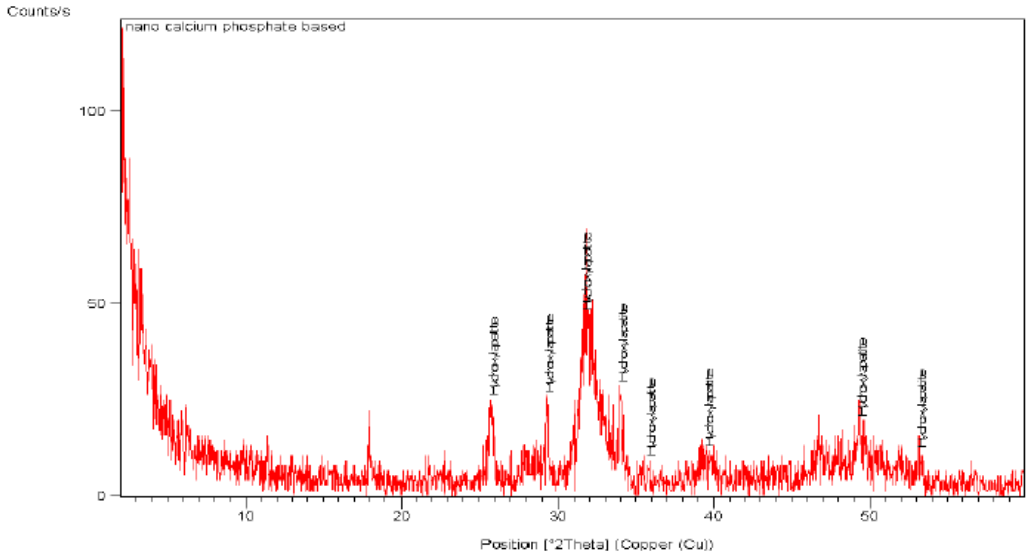

Figure (2):- XRD analysis pattern of the preparedhydroxyapatite nanoparticles.

Scanning Electron Microscope (SEM) and Energy Dispersive X-ray (EDX) Analysis results:
Analysis of the SEM micrographs presented in Figure (3a) shows agglomerates of the preparedhydroxyapatite; almost equal in size with a size range between 30nm and 100nm. Individual fine particles withcuboid and hexagonal shapes were also observed .

EDAX analysis of the preparedhydroxyapatite nanoparticles is shown in Figure (3b). The elemental analysis of the prepared particles as revealed by the EDAX spectra indicated that the particles constitute mainly of calcium and phosphate groups. The weight percentages of Ca, Pand O were found to be 17.46%, 16.03% and 66.3%, respectively.

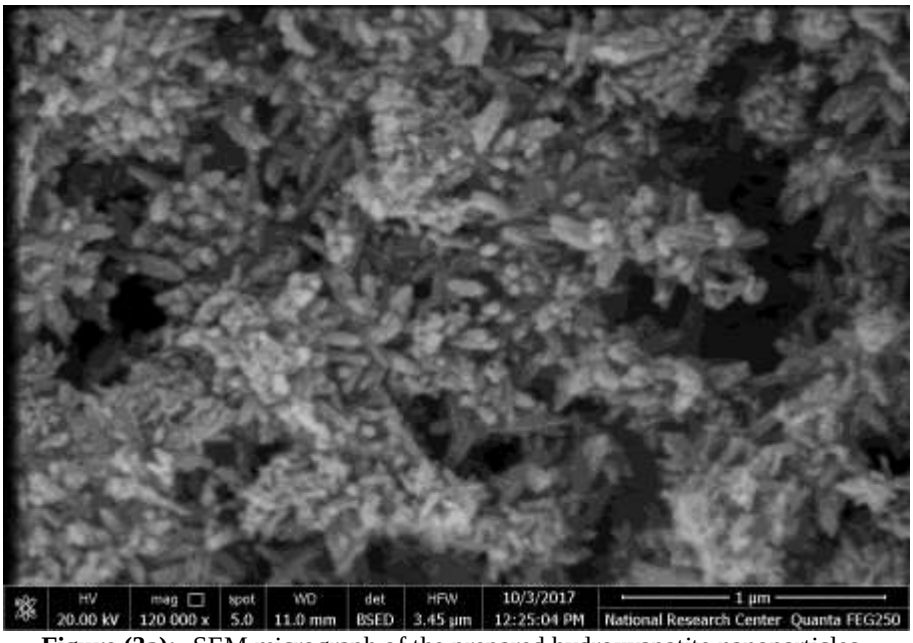

Figure (3a):- SEM micrograph of the prepared hydroxyapatite nanoparticles.

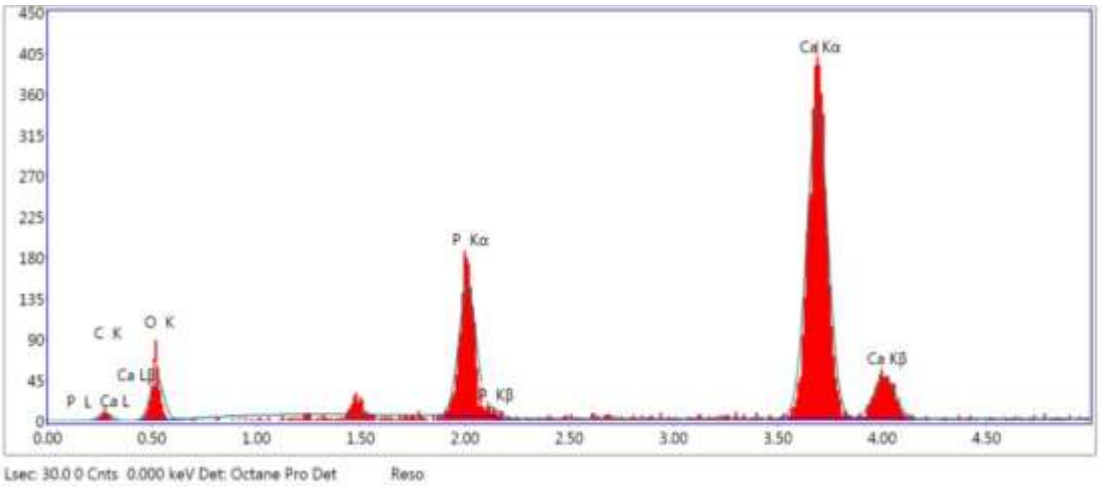

Figure (3b):- EDAX analysis of the prepared hydroxyapatite nanoparticles.

Transmission Electron Microscope (TEM) results:

TEM micrograph of the prepared hydroxyapatite nanoparticles is shown in figure (4).The morphology of the prepared hydroxyapatite appeared to be in nanostructure uniform hexagonal shaped rods

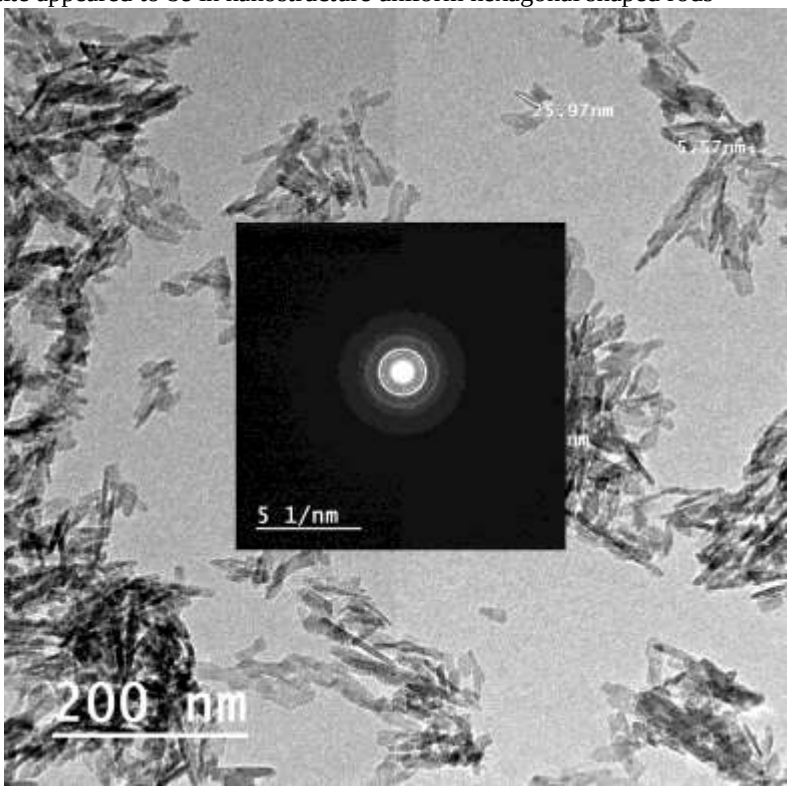

Figure (4):- TEM micrograph of the preparedhydroxyapatite nanoparticles.

Solubility testing results:

Minimum, maximum, mean and standard deviation of MTA, bone cement and bone cement with nano filler solubility were presented **in table (1)** and shown in **figure (5).**

Comparison between different groups was performed by using One Way ANOVA test which revealed a significant difference between as the three tested groups($P<0.001$), Tukey`s Post Hoc test for multiple comparisons revealed that MTA (GroupI) had the significantly lowest mean value ; having (0.010g/mm^3), while Bone cement with nanofiller (Group III) had thesignificantly highestmean value (1.009 g/mm^3).

Table (1):- Minimum, maximum, mean and standard deviation of solubility of MTA,bone cement and bone cement with hydroxy apatite nano filler and comparison between them using Independent t test:

Solubility (g/mm^3)	Min (g/mm^3)	Max (g/mm^3)	M	SD	One Way ANOVA test	
					Df	P value
MTA(GroupI)	0.009	0.011	0.010	0.001	2	<0.0001*
Bone cement	0.570	0.780	0.657	0.075		
Bone cement with nanofiller(Group III)	0.870	1.120	1.009	0.079		

Min: Minimum Max: maximum M: mean SD: standard deviation
*Significant difference as P<0.05

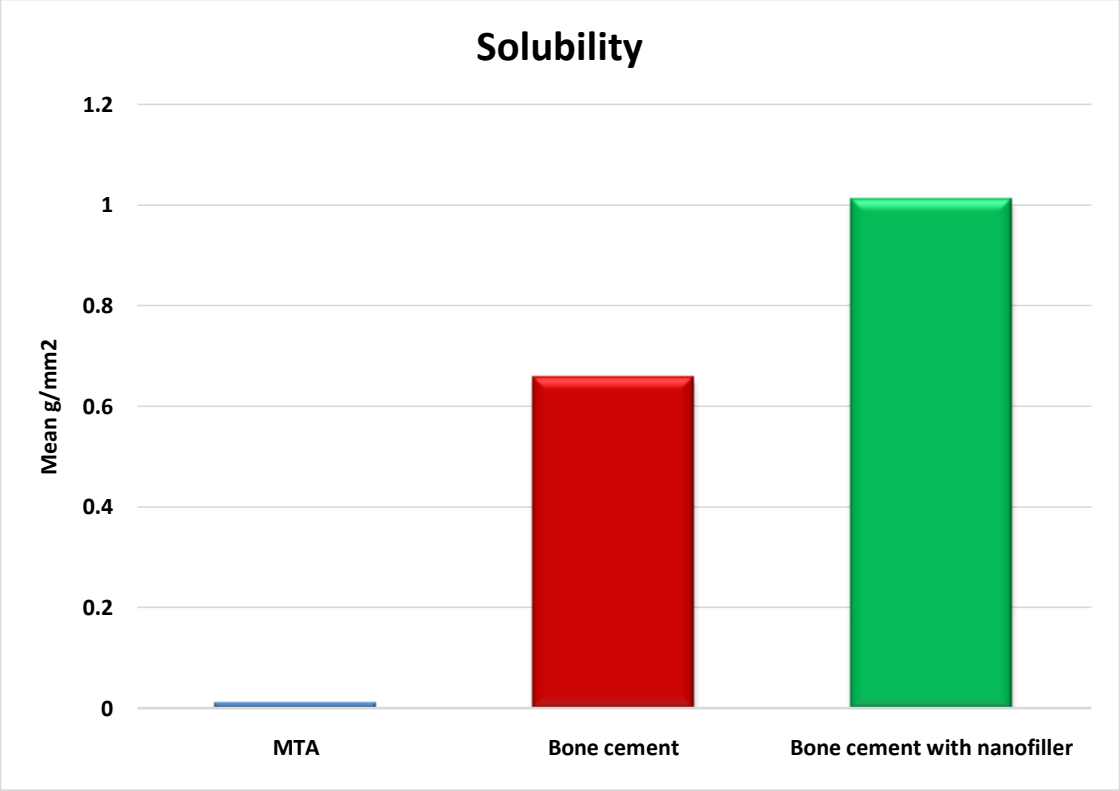

Figure (5):- Bar chart showing mean values of solubility results of MTA, bone cement and bone cement with calcium hydroxyapatite nano filler.

The biomineralization ability results:
SEM micrographs of the tested materials after immersion in SBF for 21 days are shown in figure (6). SEM micrograph of MTA and PMMAcomposite samples revealed clusters of spheroidal aggregates on the surface. No surface precipitates were detected at the surface of the PMMA bone cement sample.

The mean Ca/P ratio results was 1.76 for MTA group (group I) , zero for PMMA group (group II) and 2.83 for PMMA composite group (group III)

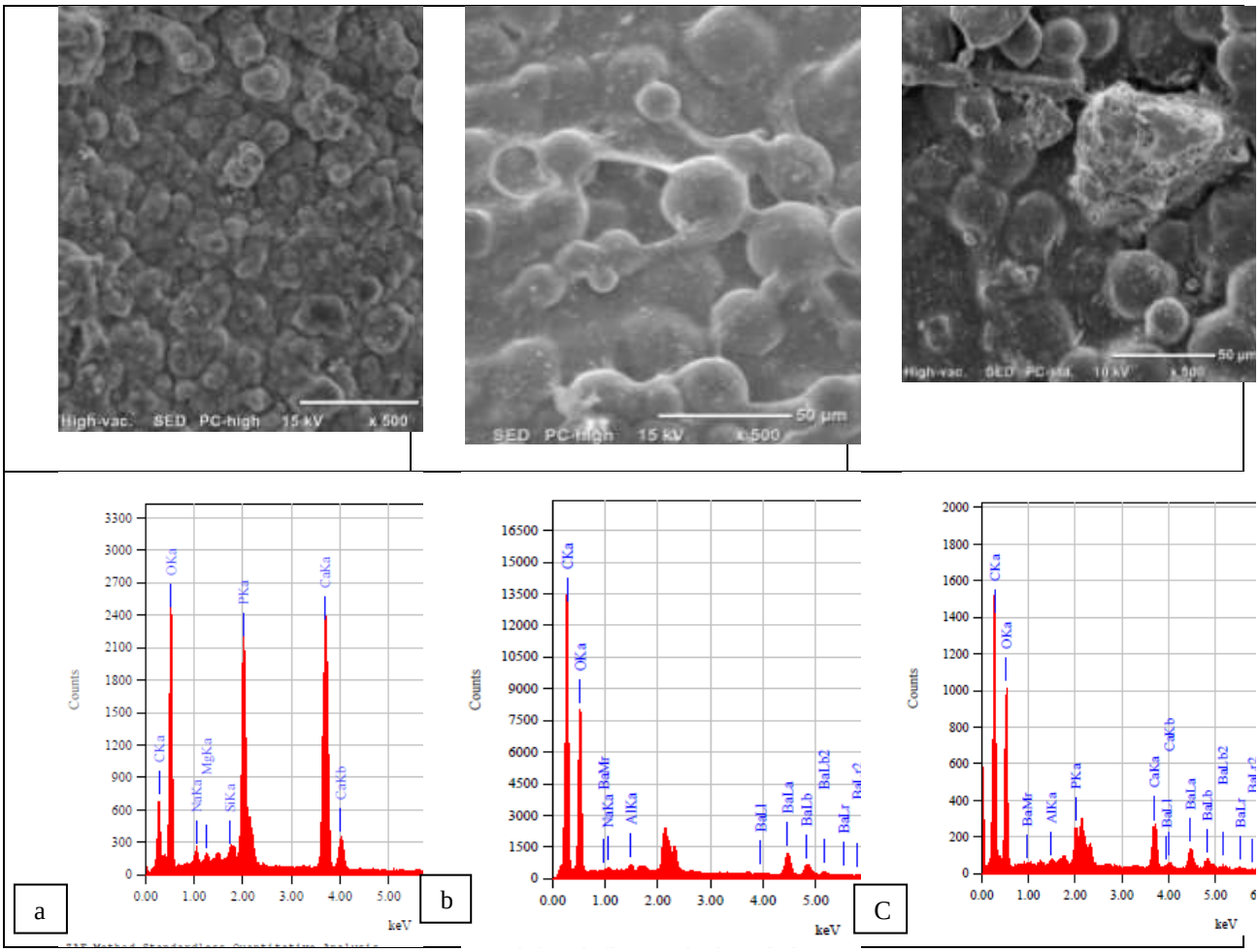

Figure (5):- SEM micrographs and EDX analysis of the tested groupsafter immersion in SBF for 21 days : a) MTA sample (x500) b)PMMA bone cement sample and c) PMMA composite sample.

Discussion:-

One of the important criteria of root-end filling materials is their insolubility since the root-end filling is in direct contact with fluids, acids and enzymes at the peri radicular tissue.[21].

The need for novel materials, with more acceptable properties for use as root end filling material has led many researchers to investigate new polymer-based composites to achieve materials with higher strength and biocompatibility. One of the tried materials is the poly (methyl methacrylate) (PMMA) used as cement. PMMA bone cementcomposed of two components, (a) a liquid methyl methacrylate (MMA) monomer, N,N-dimethyl-p-toluidine (DMpT) as activator and hydroquinone (HQ) as inhibitor and (b) a powder component composed of PMMA , benzoyl peroxide (BPO) to initiate the polymerization process.

The present study aimed to evaluate the effect of addition of the prepared nanohydroxyapatite filler to polymethyl-methacrylate bone cement on solubility and biomineralization ability and compare it with mineral trioxide aggregate(MTA) as root-end filling materials.

According to ANSI/ADA Specification No 57 of endodontic sealing material (2000); “solubility is the loss of mass during a period of immersion in water following setting and should not exceed 3% of the initial mass”[14].

In the present study, the significantly lowest mean solubility value of group I (MTA) compared to other groups may be due to the known bioactive properties of MTA in an oral environment or in contact with SBF solution with major

cationic ions release predominantly Ca resulting in precipitation of hydroxyapatite on sample surface increasing its weight.[20]

The solubility results of PMMA bone cement loaded with nanohydroxyapatite could be attributed to the fact that bone cement could absorb water or tissue fluids and release unreacted monomers, initiator, catalysts, etc, soluble in the aqueous media. The high percentage of nanohydroxyapatite fillers, the lack of bonding of fillers and polymeric matrix, and the leaching out of the calcium and phosphorous components probably could affect solubility results[19]. Results of the present investigation is in agreement with the findings of **Faria-Junior et al (2013)[10]**and **Edwards and Thomasz** (1981)[1] who reported that MTA had the lowest solubility compared to other root end filling materials.

In the present study, **scanning electron microscope (SEM)** and **energy dispersive X-ray analysis(EDX)**were used to evaluate the biomineralization ability of the tested materials and their ability to form apatite precipitates.

Simulated body fluid (SBF)having an ionic concentration similar to that of blood plasma was chosen in this study; to simulate the in-vivo conditions in the oral cavity in which root-end filling materials are used[4].

Apatite is mainly formed due to the release of calcium into biologic fluids[4]. Bioactivity or bone-bonding is the ability of the material to form a biologically active apatite layer on its surface when implanted in the human body[15].

In this study, regarding MTA samples, multiple clusters (aggregates) of spheroidal bodies (spheriolites) randomly distributed on the cement surface were observed by SEM. These findings were in accordance with the findings of **Abu Zeid et al, (2018)[1]**. Visible patches were also observed; via SEM analysis; on the surface of the samples of PMMA bone cement loaded with the nanohydroxyapatite fillers; indicating a bioactive behavior of the tested materialinduced bythe nanohydroxyapatite content. Such finding was confirmed by the SEM results. On the other hand, regarding **PMMA bone cement** samples, no precipitate could be noticed on the surface of the samples.

In this study, Scanning electron microscope (SEM) observations were in line with the EDX spectroscopic analysis. Both MTA and PMMA bone cement loaded with nanohydroxyapatite fillers showed the ability to form calcium phosphate deposits after being immersed in simulated body fluid (SBF). Calcium phosphate deposits are of clinical importance creating a favorable environment for stem cells and osteoblast differentiation and for colonization, thus supporting new bone formation[12]. In this study, regarding both MTA and PMMA bone cement with nanohydroxyapatite; EDX analysis revealed high intensity peaks for Ca and P which may indicate the precipitation of calcium phosphate deposits[12]. The Ca/P atomic ratios of precipitates presented after 21 days of immersion in SBF, both for MTA and PMMA bone cement loaded with nanohydroxyapatite fillers (1.76 and 2.83, respectively), indicating the precipitation of different calcium phosphate compounds on their surfaces. calcium phosphate deposition on the cement surface however is an indication of the bioactive behavior of the tested materials.[12]. Such findings were in agreement with **Guimares et al (2017)[12]** , **Bird et al (2012)[4]** and **Oliveira et al (2013)[16]** who reported the ability of MTA to form calcium phosphate deposits after being immersed in SBF which may be attributed to the dissociation of calcium hydroxide of MTA in SBF and its reaction with phosphorus ions of SBF solution. Also, these findings were in accordance with **Ohtsukiet al (2001)[15]** and **Renteria-Zamarronet al (2009)[18]** who reported that the modification of PMMA bone cement with calcium salts and alkoxysilanes or with more than 20 wt% wollastonite ceramics (silica) can allow the PMMA bone cement to form an apatite layer; thus rendering the PMMA bone cement from non bioactive material into a bioactive material that can bond to bone through this formed apatite layer.

Conclusion:-

Within the limitations of the present study, it can be concluded that addition of 40 wt% nanohydroxyapatite to PMMA bone cement enhanced its biomineralization ability, but increased its solubility. Solubilty of the tested composite cement is higher than that of the tested MTA .

Further researches and in vivo studies should be conducted on the prepared PMMA bone cement loaded with nanohydroxyapatite as a developed material to evaluate its clinical efficacy.

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