

Synthesis and structural properties characterization of Ap/MgO nanocomposites for biomedical applications.

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Abstract

In this study, a novel method was used to produce a nanostructured composite consisting of hydroxyapatite and MgO by varying the temperature. The structure and morphology of the synthesized nanocomposite were characterized. From x-ray diffraction (XRD) analysis, an increase in crystallite size and degree of crystallinity with an increase in the temperature was observed. The XRD investigation confirms that the grain size of the synthesized nanocomposites is in the range 4-11 nm. The grain size increases when the temperature is increased due to the agglomeration of nanoparticles. The fourier transform infrared spectroscopy (FTIR) studies confirm the presence of PO_4^{3-} , CO_3^{2-} and Mg^{2+} , and OH^- groups. The UV-VIS spectroscopy measurements show the reduction in the band-gap upon the temperature. Transmission electron microscope (TEM) images ascertained that nano HA_p/MgO at 250°C and 300°C composite shows flakes like morphology.

Keywords: Nanocomposites, Hydroxyapatite, MgO, TEM, X-ray diffraction.

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Introduction

Among all calcium phosphate bioceramics, hydroxyapatite (HA_p), $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, is the most extensively used biocompatible ceramic materials for bone tissue engineering, as its chemical composition is similar to the bone mineral phase. Biological apatites are non-stoichiometric nanocrystal-line carbonated Has (CHA). Therefore, synthetic HA_p ceramics are doped with small amounts of additives (e.g., Mg_2O , Zn_2O , Mn_2O ions). Although the substitution does not intensely change the crystallographic properties of HA_p , it affects the biological and mechanical properties [1-4]. An ionocovalent structural model of the apatite family shows a structure that can accept both cationic and anionic substituents. These substitutions induce modifications in lattice parameters and crystallinity, which substantially influence the solubility of HA_p at physiological conditions without generating significant changes in the hexagonal system of apatite. One of the elements associated with biological apatite is magnesium. Mg incorporation into HA_p stimulates osteoblast proliferation. Mg acts similar to a growth factor during the early stages of osteogenesis and promotes bone formation. Typical concentrations of carbonate and Mg ions in human bone are 5.8 and 0.55wt%, respectively.

Although the extent of these elemental substitutions is minimal, they are important for biological activity and interaction between bone mineral and calcium-phosphate-based implant materials by influencing crystal growth, dissolution rate, solubility, surface chemistry and charge, morphology, and the mechanical properties. By substitution of a smaller Mg ion for a larger Ca ion, additional structural changes may be required to prevent destabilization/decomposition of the structure during heat treatment process. This can be achieved by co-

substitution of a second ion, to the HA_p structure [5-7]. Thus, MgO nanoparticles dispersed within polymer composites have the potential to enhance bone tissue formation with limited adverse degradation reactions. Taking advantage of these prior studies, the objective of the present *in vitro* study was to characterize MgO nanoparticles as additive materials for orthopedic tissue engineering applications, especially when used in combination with HA_p nanoparticles.

Materials

HA_p/MgO nanocomposite was prepared using a novel environmentally benign technology called a hydrothermal method. The following precursors were used for the preparation of nanocomposite. Calcium Nitrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, Merck Gd, 99%) is taken as the Calcium precursor. Diammonium Hydrogen Phosphate (NH_4HPO_4 , Merck Gd, 99%) is taken as the Phosphate precursor. Magnesium Nitrate hexahydrate ($\text{Mg}(\text{NO}_3)_2 \cdot 9\text{H}_2\text{O}$, Merck Gd, 99%) is taken as the Magnesium precursor. NaOH (Merck, purity 99%) is taken as the pH reagent. Double distilled water is used to obtain a homogeneous solution.

Synthesis of nano HA_p/MgO composites

The 0.1 M of $\text{Mg}(\text{OH})_2$ was prepared by adding 6.41 g of Magnesium Nitrate dissolved in 100ml de-ionized water. Then the 0.8 g of NaOH solution was prepared in 50 ml de-ionized water. The NaOH solution added to increase the pH value to 11. Thereafter the prepared solution was allowed for overnight stirring and kept undisturbed for aging.

In order to synthesize nano-hydroxyapatite 5.9 g of Calcium Nitrate and 1.98 g of Diammonium Hydrogen Phosphate were taken in the hydrothermal vessel and mixed with de-ionized

water with the molar ratio of 1:0.6 to maintain the Ca/P ratio 1.67 which is the stoichiometric ratio of HA. The pH of the above solution is increased to 9 by adding 0.2 M of NaOH solution in 50 ml. The prepared Mg(OH)₂ solution was added drop by drop to the above solution. The vessel is placed in the oven of a hydrothermal instrument. The mixed solution was hydrothermally treated at 250°C for 5 hours. After 5 hours the autoclave is cooled to room temperature. Hydroxyapatite-MgO nanocomposite is separated by filtration, washed a few times and kept in hot air oven at 60°C. The dried composite is grind using mortar and pestle. Similarly, the remaining sample was prepared at 300°C.

Results and Discussion

Xrd analysis

The X-ray diffraction (XRD) patterns of the powdered nanoparticles were recorded using XRD-1008 with Cu-K α radiation (1.54060Å). XRD pattern for HA_p/MgO Nanocomposite synthesised at 250°C and 300°C shows clear reflections from the (1; 1; 2), (0; 0; 2) (2; 1; 1) and (3; 1; 0) planes in the range 25°C to 50°C. The prepared samples show well-defined peaks which indicate high crystalline nature. All the diffraction peaks could be readily indexed with pure hexagonal phase (space group: P6₃/m (176)) with lattice parameters of a=b=9.460Å and c=6.880Å which are in accordance with the JCPDS file card number #821943. The average crystallite size of the sample synthesized at 250°C and 300°C as calculated by Scherrer's formula ($D=k\lambda/\beta\cos\theta$) was 12.30 nm and 15.309 nm respectively. The value of lattice constants obtained are a=b=10.405Å, c=6.4388Å and a=b=10.2499Å, c=6.3805Å. The unit cell volume (V) inferred are 1804.7671Å³ and 1735.5056Å³. 0.0085, 0.0150 are the degree of crystallinity (X_c (%)). The (0; 0; 2) peak was chosen to calculate the average crystallite size, lattice parameters, unit cell volume and degree of crystallinity for HA_p. It is known that the preferential growth of apatite crystal is along the c-axis [8,9].

The relationship between lattice constant (a and c), Miller's indices (h; k; l) and lattice spacing (d) is used to calculate lattice parameter values, expressed as:

$$\frac{1}{d^2} = \frac{4}{3} \left(\frac{h^2 + hk + k^2}{a^2} \right) + \frac{l^2}{c^2}$$

The volume (V) of the hexagonal unit cell and the degree of crystallinity (X_c) of the MgO/HA_p nanocomposites are obtained using the equation, respectively, $V = 2.589 a^2 c$ and (Figures 1a and 1b).

$$X_c = \left(\frac{K_A}{\beta} \right)^3$$

Where, β is the full-width at half maximum, X_c the degree of crystallinity, K_A the constant (0.24) and a and c are lattice parameters [10].

FTIR analysis

The characteristic peaks of nano HA_p/MgO composite were examined by Fourier Transform Infrared Spectroscopy (FTIR). The pellet for FTIR measurements was prepared by mixing the sample (2 mg) with 200 mg of IR grade KBr. The mixed powder was pressed in a stainless steel pellet dye for one minute at constant pressure to obtain a translucent disc. The absorption spectra were measured at 4000-400 cm⁻¹ using SHIMADZU spectrometer, Japan.

Biological HA_p is usually Calcium deficient; it is always substituted with a carbonate (Figures 2a and 2b).

The most characteristic peaks in the FTIR spectra of synthesized HA_p/MgO nanocomposites at 250°C and 300°C are PO₄³⁻, OH⁻, CO₃²⁻ and Mg²⁺. CO₃²⁻ forms a strong peak at 1381.03 cm⁻¹ and 1396.43 cm⁻¹. The broad peaks appeared at 1018.41 cm⁻¹ and 1080.14 cm⁻¹ indicate the presence of the asymmetric stretching (ν_3) mode of vibrations because of the phosphate bonds [8,9]. Weak peaks in the range 550-620 cm⁻¹ are observed due to the presence of Mg²⁺ ions. The broad peak spread over the range between 2957-3450 cm⁻¹ and 1627.92 cm⁻¹ reveals the superposition of absorption because of the stretching mode of surface hydroxyl groups and adsorbed water molecules [10]. OH⁻ group forms strong and broad peaks in the range 2900-3410 cm⁻¹ shown in (Table 1).

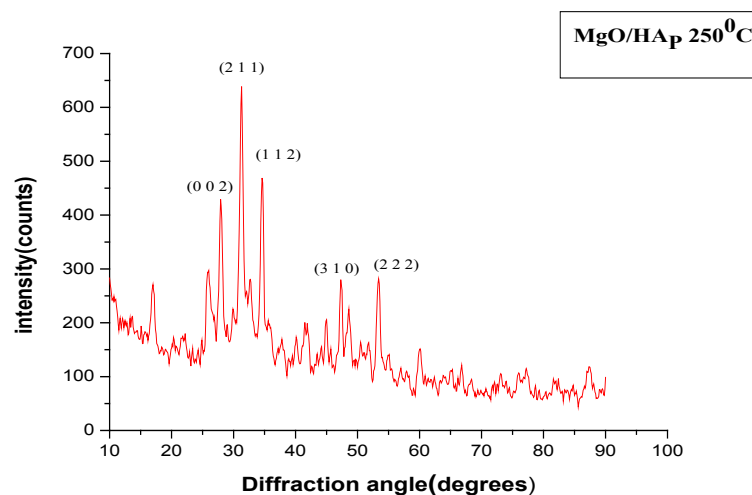


Figure 1a. XRD pattern of HA_p/MgO nanocomposites at 250°C.

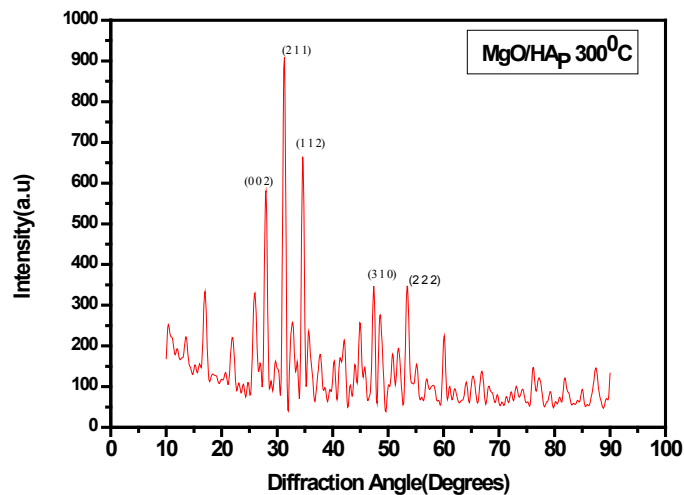


Figure 1b. XRD pattern of HA_p/MgO nanocomposites at 300°C.

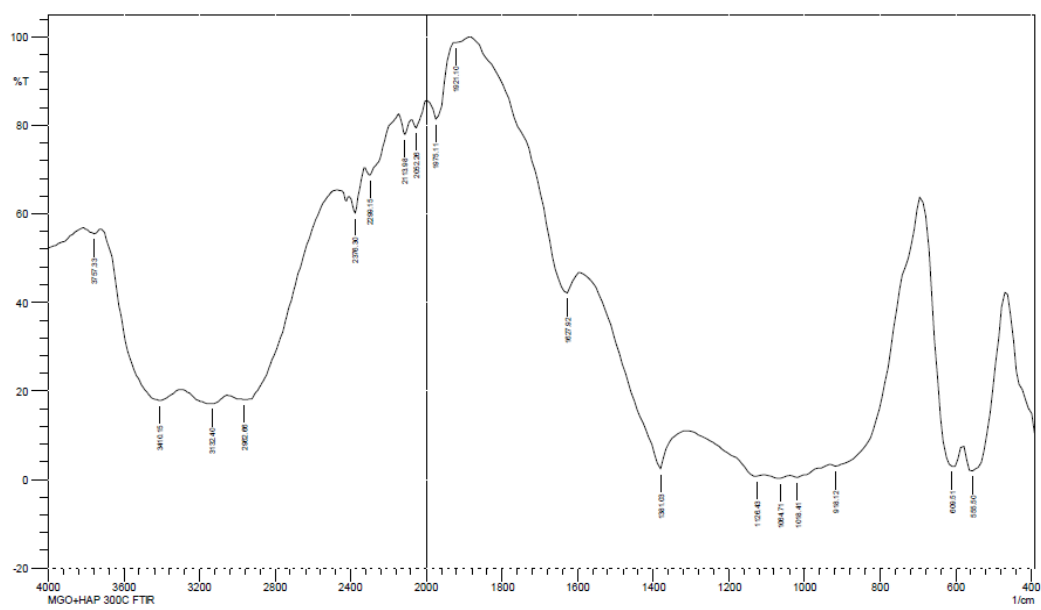


Figure 2a. FTIR pattern of HA_p/MgO nanocomposites at 250°C.

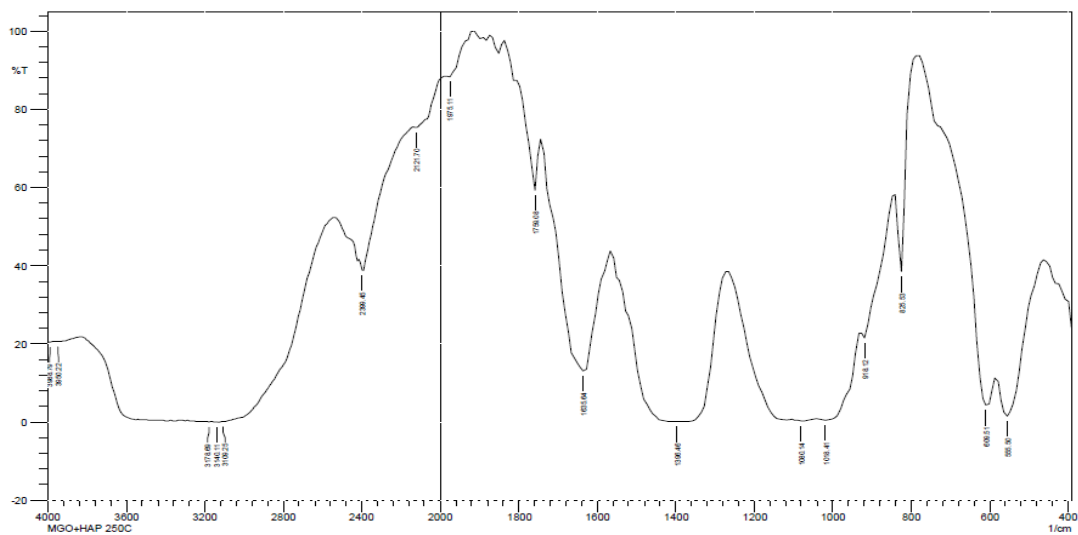


Figure 2b. FTIR pattern of HA_p/MgO nanocomposites at 300°C.

UV-VIS spectrum analysis

The UV-visible absorption spectra of the samples were recorded in the wavelength range of 200 nm to 800 nm using a Shimadzu UV 1700 UV spectrometer. The band gap energy was calculated by using Einstein formula ($E=hc/\lambda$). Where h is a Planck’s constant (6.626×10^{-34} J), c is the velocity of light (3×10^8 m/s) and λ be the wavelength of the sample recorded.

Table 1: FTIR spectrum analysis.

Vibrational Frequency (Cm ⁻¹)	Assignments
HA_p/MgO nanocomposites at 300°C	
1381.03	Carbonate band
1018.41, 1064.71	v ₃ asymmetric stretching of phosphate
555-610	v ₄ fundamental vibrational mode and bending vibration of the PO ₄ ³⁻ ions
2960-3410	Stretching mode of surface hydroxyl Groups and adsorbed water molecules
HA_p/MgO nanocomposites at 250°C	
1396.43	Carbonate band
1018.41, 1080.14	v ₃ asymmetric stretching of phosphate
555-610	v ₄ fundamental vibrational mode and bending vibration of the PO ₄ ³⁻ ions
3100-3200	Stretching mode of surface hydroxyl Groups and adsorbed water molecules

UV-visible spectroscopy was used to characterize the optical absorption properties of HA_p/MgO nanocomposite at 250°C and 300°C. We obtained the band gap to be 5.97eV for MgO/HA_p at 300°C and it starts decreasing for MgO/HA_p at 250°C as 5.689eV respectively. The decrease in Eg for decreasing the temperature (Figures 3a and 3b).

TEM analysis

Transmission Electron Microscope (TEM) is an equipment to let the incident electron beam to transmit a thin specimen at high acceleration voltage, 80-3,000 kV, which results in generating signals caused by the interaction between the specimen and incident electrons. Structures, compositions and chemical bonding of the specimen can be determined from these signals.

The particle size and morphology of the synthesized nano HA_p/MgO composites with different temperature have been examined by TEM. Figures 4a and 4b reveals the TEM images of HA_p/MgO nanocomposite at 250°C and 300°C respectively. The sample exhibits relatively flakes like morphology [11,12].

Conclusion

In this study, nano HA_p/MgO composites were successfully prepared at 250°C and 300°C using the hydrothermal method. The crystallite size, crystallinity, functional groups and morphology, were studied using XRD, FTIR, UV-visible absorption measurement, and TEM analysis. The XRD investigation confirms that the grain size of the synthesized

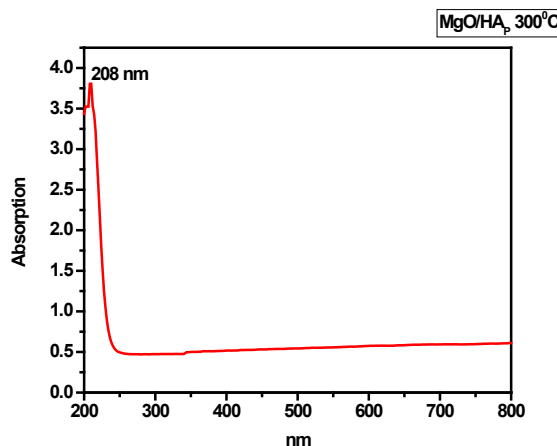


Figure 3a. UV-VIS spectrum of HA_p/MgO nanocomposites at 300°C.

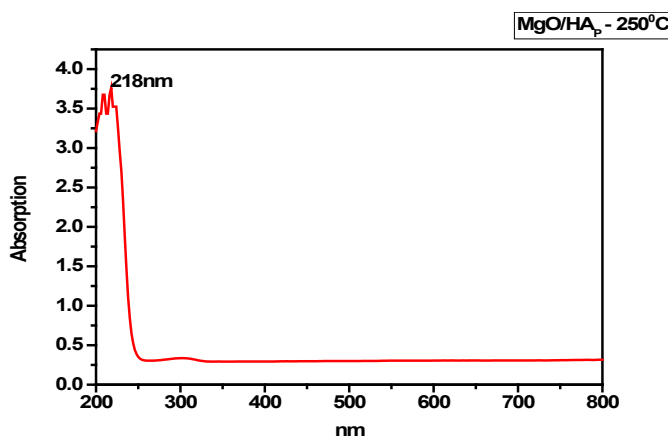


Figure 3b. UV-VIS spectrum of HA_p/MgO nanocomposites at 250°C.

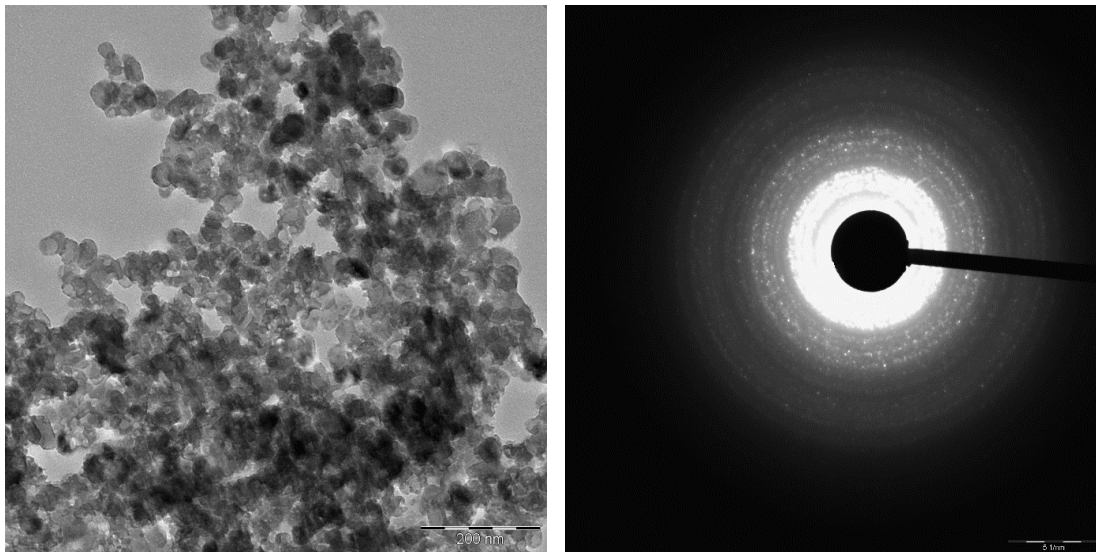


Figure 4a. TEM image (b) diffraction pattern of MgO/HA_p at 250°C.

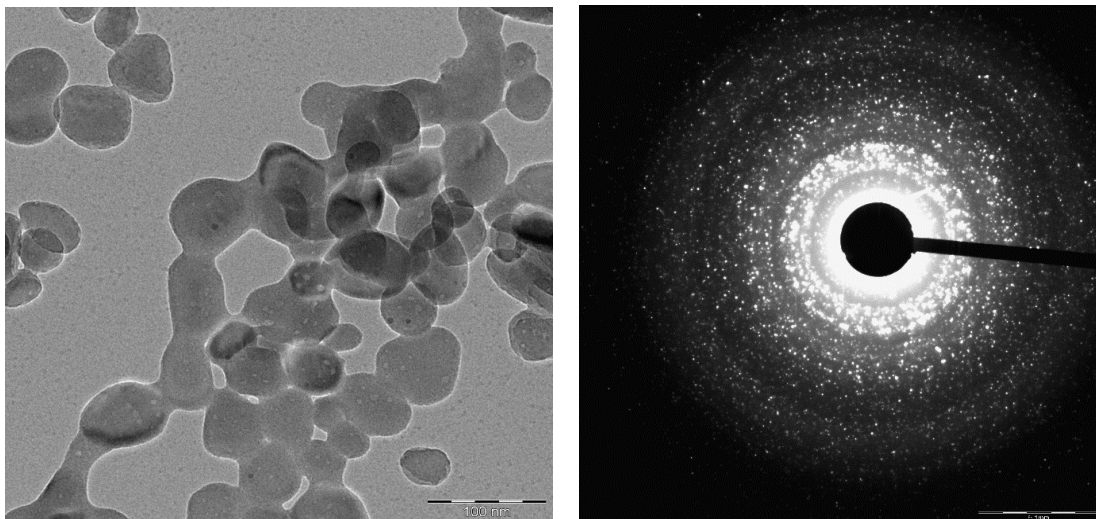


Figure 4b. TEM image (d) diffraction pattern of MgO/HA_p at 300°C.

nanocomposite is in the range 4-11 nm. The value of lattice constants obtained are $a=b=10.405\text{\AA}$, $c=6.4388\text{\AA}$ and $a=b=10.2499\text{\AA}$, $c=6.3805\text{\AA}$. The unit cell volume (V) inferred are 1804.7671\AA^3 and 1735.5056\AA^3 . 0.0085, 0.0150 are the degree of crystallinity (X_c (%)). The hexagonal phase with space group $P6_3/m$ (176) has been confirmed from XRD results. The grain size increases when the temperature is increased due to the agglomeration of nanoparticles. The FTIR studies confirm the presence of PO_4^{3-} , CO_3^{2-} and Mg^{2+} , and OH^- groups. The UV-VIS measurements show the reduction in the band-gap upon the temperature. We obtained the band gap to be 5.97eV for MgO/HA_p at 300°C and it starts decreasing for MgO/HA_p at 250°C as 5.689eV respectively. TEM images ascertained that nano HA_p/MgO at 250°C and 300°C composite shows flakes like morphology. This study provides a platform for further research on the HA_p/MgO nanocomposites for biomedical applications.

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