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Research Article

EFFECT OF HIGH ENERGY BALL MILLING GRINDING ON PHYSICO-CHEMICAL, STRUCTURAL AND MORPHOLOGICAL STUDIES OF BITTER MELON (*MOMORDICA CHARANTIA*) NANOPOWDER

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ABSTRACT

Bitter melon is used as a traditional medicine for several indispositions such as diabetes, abortifacient, anthelmintic, contraceptive, dysmenorrhoeal, cancer etc. and it mainly comprises of protein, steroid, alkaloid, phenols, lipid, triterpene, and inorganic compounds. Green synthesis method is used to synthesize Bitter melon nanometric size -powder with the use of high energy ball mill equipment. These synthesized nanopowders were then characterized by X-ray diffractometer (XRD), Scanning Electron Microscope (SEM) for structural analysis, Fourier Transform Infrared (FTIR) Spectroscopy for chemical nature of bond, UV/VIS/NIR spectrophotometer, Photoluminescence (PL) for electronics structure cum luminescence, Vibrating Sample Magnetometer (VSM) for magnetic behavior. These food powders in nanometric size may improve the physicochemical properties of food materials and can be used for biomedical applications as well as in the pharmaceutical industry.

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INTRODUCTION

Material Science and nanotechnology is the new elixir to human life and it is influencing all aspects of human living including food science. Food technology is appreciably influenced by nanotechnology and it helped us in achieving new and improved properties of different food materials due to its different sizes and powder form. New applications of these nanoparticles are based on their new chemical and physical properties, like solubility, absorption behavior and their action interaction. Green synthesis of nanoparticles is also facilitating the manufacturing of food based products that are eco-friendly, safer and have sustainable viability (Manjule *et al*, 2012; Deveswaran *et al*, 2010). The plant based extracts usage in pharmaceutical industries is increased in recent years because they are biocompatible, less expensive, non-toxic and have minimal chances of side-effects (Parekh *et al*, 2006). In today's world a significant number of plant extracts have met the requirements for drug delivery systems and they are competing with the synthetic excipients. *Momordica charantia* is a common vegetable in Asian country and has lots of medicinal properties. It belongs to the family cucurbitaceae. The bitter flavor/taste of this fruit is due to alkaloid momordicine commonly known as Bitter melon/Bitter guard in Indian subcontinent (Parekh *et al*, 2006; Bagchi, 2005; Ariyawansa

2011). Also it has a complex array of bioactive chemicals, Vitamins, minerals and antioxidants and has lots of medicinal benefits (Joseph, 2013; Bakare, 2010). It is commonly used as an anti-diabetic agent (Kaur *et al*, 2016; Talreja *et al* 2015). Bitter melon or bitter gourd and has recently attracted considerable attention for its various physiological activities, such as its antitumor, anti-inflammatory, antioxidant, anti-bacterial, antifungal, hypoglycemic, hypocholesterolemic, hypotriglyceridemic, and immune stimulating activities (Singh *et al*, 2006; Jiratchariyakul *et al*, 2001; Prabhu *et al*, 2015; Puri *et al*, 2009; Natesan *et al*, 2008; Roopshree *et al*, 2008; Khan *et al*, 1998; Kumar *et al*, 2010; Tan *et al*, 2015; Giron *et al*, 1991; Shaikh, 2016). Grinding these bitter melons powder with the help of high energy ball mills can make these powder to superfine ones and may improve the physicochemical properties. During the milling process these powders undergo structural changes and exhibit better properties than their bulk form. The beneficial properties of this powder are attracting many researches in this area since last decade. It is useful in diseases such as diabetes, cholera, bronchitis, anemia, blood related diseases, skin problems, ulcer, diarrhea and dysentery. It can be also used as a cure for gonorrhoea (Gupta *et al*, 2011). The present work is designed to get the insight of the change in crystalline size, functional group, optical and magnetic properties after different hours of

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milling. This in depth information about the physicochemical properties of superfine powders promotes its usage in the food science and pharmaceutical industry (Aschberger *et al* 2015). These superfine powders were characterized by modern scientific tools. According to the literature survey, this is a novel work because the synthesis and characterizations of nano powders of bitter-melon is not reported to the best of my knowledge. However there are few works on using Bitter Melon powder as reducing agent to synthesize metal nanoparticles. Application work is in progress, which is our future research.

MATERIALS AND METHODS

Bitter melon fruits powder was purchased from the Prajakta Technology Pvt. Ltd, Pune Maharashtra, India. Then the superfine powder was made using High Energy Ball Mill (Emax Retsch, Germany). The powder sample was put in 50ml stainless steel jar with 2mm steel balls. The weight of balls to the Bitter melon powders was kept as 20:1 ratio and the milling conditions were kept as at 500 rpm for 5 hour and 10 hours. The rotational direction of ball milling was changed every 30 min. The ball-milling process was carried out at 27°C and the temperature was maintained by the air cooling system to prevent overheating. After the treatment, the samples were sealed in a bag for analysis. All the analysis was carried out in powder form.

The 2θ range varied from 10 degree to 80 degree at 2°/min scan rate. The sample was kept on sample holder within the chamber of analytical X-ray having wavelength 1.54 Å of CuKα radiation. The XRD data observed for general Bitter melon powder, milled for 0hr, 5hr and 10hr are shown in figure 2. Crystalline sizes of the synthesized powders were calculated using Scherrer's formula.

$$d = K\lambda / \beta \cos \theta$$

Where K is a constant that has value 0.9, β (FWHM) is equal to twice the diffraction angle θ and λ is the wavelength of Cu-Kα radiation ($\lambda = 1.54056 \text{ \AA}$).

The smoothed resultant diffractograms by using the Origin 8.0 software (Originlab Corporation, Northampton, USA) and then finally calculated the relative crystallinity. The crystallite size for four most intense intensity peaks found to be between 9nm to 40 nm shown in the table 1.

Table 1 Angular position and crystalline size obtained from XRD pattern

Angle(2θ)	0 Hr.	05 Hr.	10 Hr.
11.8	48nm	35nm	31nm
18.9	35nm	35nm	28nm
21.1	48nm	41nm	31nm
26.5	26nm	49nm	28nm



Figure 1 Bitter Melon Plant, Bitter Melon Fruits and Bitter melon NanoPowder.

Structural and Microstructural Studies

X ray diffraction studies

X-ray diffractometer (D8 Advance, Bruker, Germany) is used to determine the average crystalline size and structural properties of Bitter melon powder synthesized by E_{max} ball milling. XRD was operated at 40kV, 40mA and 25°C.

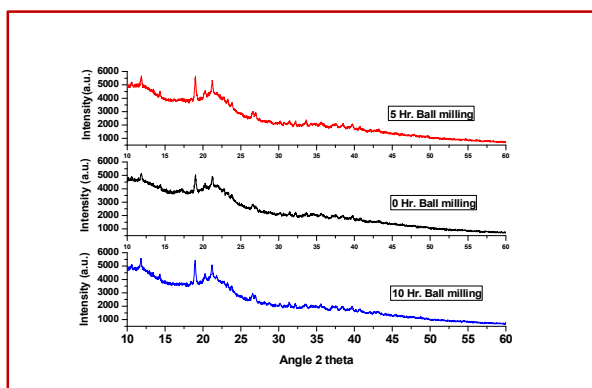


Figure 2 XRD Pattern of Bitter Melon

The prominent peak positions are at 11.8, 18.9, 21.1 and 26.5 angular positions (2θ) respectively for the three samples and the average sizes are 48nm, 35nm, 48 nm and 26 nm respectively. It revealed that the crystallite sizes of all constituents are in nanometer range, and there is a little reduction in size with increase in milling hours. Thus XRD study revealed that particle size, crystallinity and peak intensity height changes of different samples. The different intensity peak position and interplaner distance (d), and also confirms the change of size of powder with different degree of crystallinity. Further to know the grain size and morphology SEM analysis is given in figure 3.

Scanning Electron Microscopy Measurement

The Scanning electron microscope (EVO 18, Research, Zeiss, UK) images were taken at 20kV EHT with 9 mm working distance. The powders were coated with gold and palladium mixture in spin coater and then the images were taken. SEM study showed that the change in original structure of bitter melon powder. The SEM analysis of bitter melon powders it was observed that the powders are showing coalescence. The

mean grain size of this powder is in nanometer for all three milling conditions. The particles are irregular in shape but are in nanometric range.

can say that there is a structural change due to grinding in the ball milling. The FTIR (Figure 4) of the isolated substance showed the presence of functional groups like 3400

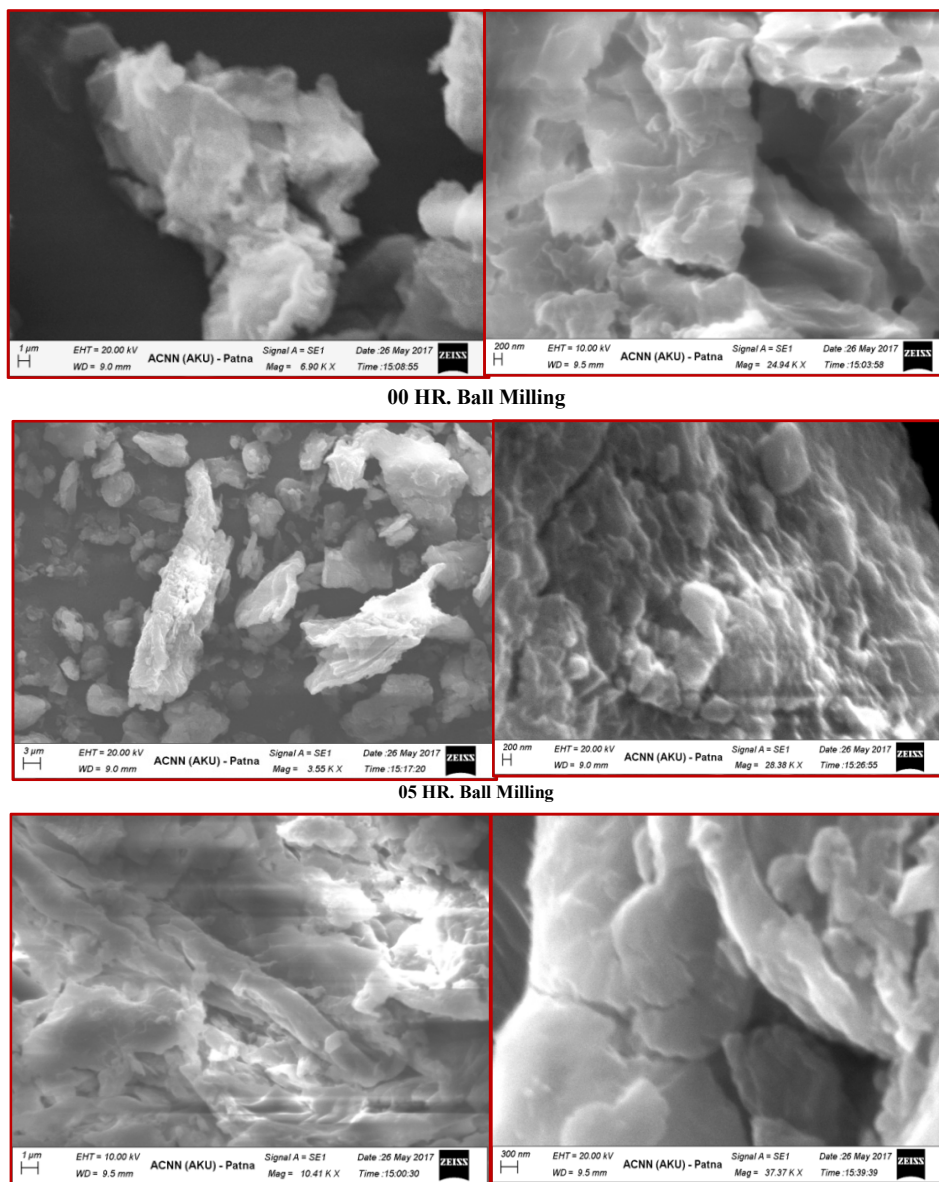


Fig 3 SEM images and Grain size distribution of powder

SEM study showed the variation in internal structure during milling for different time intervals and as a result, degree of crystallinity and amorphism is found to change as it can be clearly noticed shown in XRD pattern. Such changes were also observed in other food materials e.g. curcumin, ginger etc (Aman *et al*, 2018; Ming *et al*, 2015; Shenghua *et al*, 2014).

FTIR Study

Bitter melon powders were prepared by the pellet method with potassium bromide (KBr). Spectra were observed with FTIR (Frontier, PerkinElmer, Germany) at room temperature. Figure 4 shows the FTIR spectra of ball milled Bitter melon powders at three different durations (a) 0 h (b) 5 h (c) 10 h. The FTIR spectra are recorded from wave number 400 cm^{-1} to 4000 cm^{-1} at room temperature. Small peak shifts were observed after milling. Peak position at 536 cm^{-1} is common in 0 hr & 05 hour. There were additional five peaks that formed during the ball milling. These were 1324, 1151, 1103, 862 and 619 cm^{-1} . So we

(Broad, Free OH Stretching), 1600 (C=C), 1042 (C-O str.), 860 ($>\text{C}=\text{CH}_2$). In the “fingerprint” region the intense and broad Absorption band characteristic of C-C, C-O and C-H are present. The infrared spectra of Bitter melon showed the presence of C-C stretching band at 1384 cm^{-1} , C-O stretching band at 1239 cm^{-1} . The strong peaks at 1640 cm^{-1} were assigned to the C=C stretching modes. The very weak band at 1102 cm^{-1} was assigned to the C-O stretching and the same attribution is available for the strong peaks at 1152 cm^{-1} . These peaks appeared when the samples were milled for different hours. The in-plane bending modes of C-H are attributed to the weak peak at 1384 cm^{-1} are present in all powders. The in plane symmetrical bending of C-C is present at 536 cm^{-1} present for two samples milled for 0 and 5 hours. The additional peaks formed during the ball milling were 1324, 1151, 1103, 862 and 619. It can be said that the structural changes formed due to grinding in the ball milling.

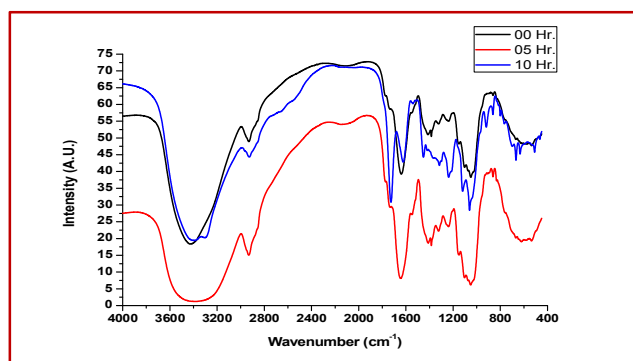


Fig 4 FTIR Spectrum

Optical properties

UV/VIS/NIR

The UV/VIS/NIR spectrum was recorded on UV/VIS/NIR Spectrophotometer (Lambda 950, PerkinElmer, UK) between wavelengths 250 to 2500 nm of all the three samples milled for different hours. The spectrum was recorded by taking the powder samples. UV/VIS/NIR Spectra showed that the Bitter melon powders absorbs at 650 and 1980nm. The increase milling hours increases the absorption in bitter melon powders. This showed the size dependent optical properties in food material which may be due to change in degree of crystallinity, different grain size distribution and types of new chemical bond shown in figure of FTIR spectrum.

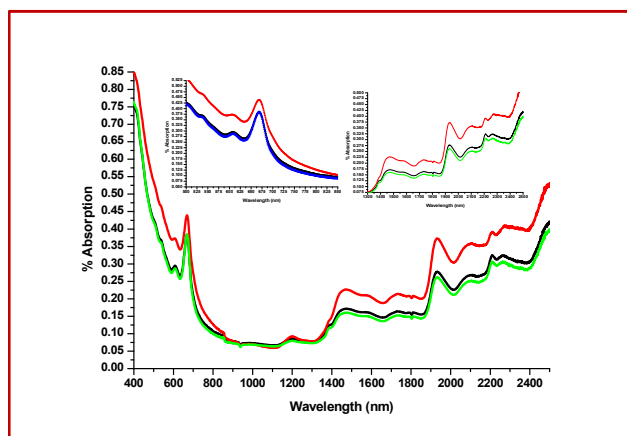


Fig 5 UV/VIS/NIR Spectrum

Photoluminescence

As the size of a particle is found different, the structural, microstructural and number of defects in a crystal per particle changes. Generally radiation grouped in to certain wavelength region including UV region, visible region and NIR region respectively. The important property of luminescence spectra is judged by peak intensity height, region of emission and brightness. The PL measurement (LS 55, PerkinElmer, Germany) of the synthesized sample is shown in figure 6. The PL spectra of milled powders are slightly different than the unmilled powder. Two peaks between 400-600nm disappeared after milling. Also, there is a slight increase in PL intensity after milling. The powders milled for 5h shows a high peak around 820nm. Such observation in luminescence is due to different electronics structure, functional groups, and

crystallographic phases as shown in XRD, FTIR and SEM images. Such types of luminescence are also observed in some food nanomaterials (Le *et al*, 2016; Mishra *et al*, 2017). Further detail mechanism of such luminescence is still required.

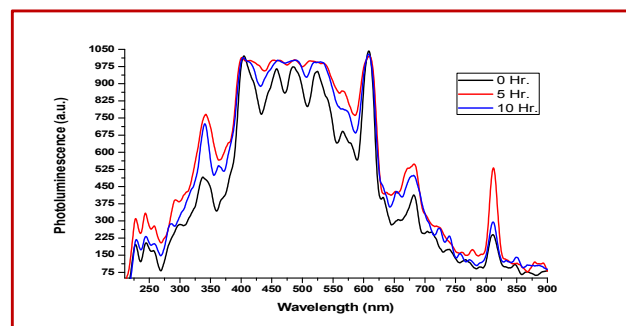


Fig 6 PL Spectrum

Magnetic Properties

The magnetism of this sample is to be measured using Vibrating Sample Magnetometer (7410, Lake Shore USA) and shown in figure 7. The magnetization in the bitter melon powders is may be due to the presence of iron or can be due to the reduction into the small fine particles using ball milling machine. There is an increase in the magnetization with the increase in milling time. It showed that the magnetic property of enhances with the decrease in size. Magnetic behavior of materials which strongly influenced by crystalline size, surface effects, environment such as inter-particle and particle-matrix interactions, Surface effects, spin canting of different grains. Milling physically can be related to the symmetry breaking of the crystal structure at the boundary of each particle. Magnetic properties enhance the medicinal value of food powders and may be used as nanomedicine. Grinding of food powder generally increases solubility of powder and absorption in human body which are beneficial for effectiveness at cellular level. Several results suggest that iron oxide Nanoparticles are a promising nanomaterial, which provide a convenient route for cellular targeting with great potential for application in various disease treatment (Singh *et al*, 2016; Singh *et al*, 2017; Chaves *et al* 2017). Further study is needed to know the cause of magnetism in this food nanomaterial.

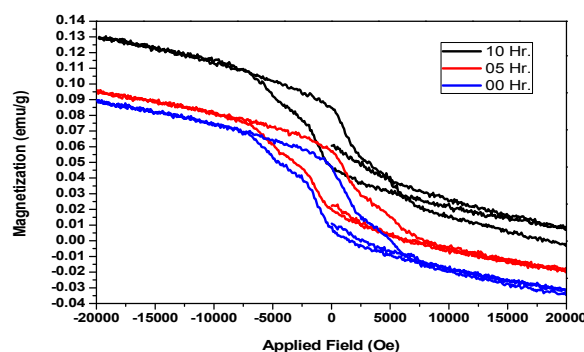


Fig 7 VSM graph of bitter melon powder

CONCLUSIONS

Nanometric size bitter melon powder of average crystalline size between 29-48nm were successfully prepared by high energy ball milling technique, and are confirmed by XRD and SEM results. FTIR spectra are almost the same but intensity of band

was found to increase for sample milled for 5 hours and decrease for sample milled for 10 hours and new peaks were found due to grinding during ball milling. The optical property of bitter melon is due to different electronics structure, functional groups, and crystallographic phases. Grinding of food powder generally increases solubility of powder and absorption in human body which are beneficial for effectiveness at cellular level. Magnetic properties enhances the medicinal value of food powders and may be used as nanomedicine and also as promising nanomaterial, which provides a convenient route for cellular targeting with a great potential for applications in various disease treatments. This was an eco-friendly and simple approach for synthesizing nano powder can be proved very effective and useful in biomedical and pharmaceutical industry.

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