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A convenient green method to synthesize β-carotene from edible carrot and nanoparticle formation

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A simple green chemistry approach was used to synthesize β-carotene from edible carrot without using solvent and heat. In this study, beta-carotene was extracted from edible carrots through a natural extraction method with several physical technical steps. The extracted sample was isolated using high-performance liquid chromatography (HPLC). A β-carotene peak was detected from HPLC with an absorbance maximum (2.135 mAU). The absorption of UV-Visible spectrum indicated that beta-carotene absorbs most strongly between 230-300 and 400-500 nm. The functional groups, namely, C=C (1649 cm^{-1}), =C-H (900-900 cm^{-1}) vibrations of antisymmetric deformation of CH_3 groups and CH_2 groups (1350-1450 cm^{-1}) of beta carotene were identified by using the FTIR test. The 537, 538, and 539 peaks for all carbon atoms (C-12), one of the 40 carbon atoms is (C-13) and two carbon atoms as (C-13) were indicated using FTMS. The resonance relates to the protons connected to methyl and methylene groups and those attributed to the vinylic protons (H-C=C) were observed between 1-2 and 5-6 ppm, respectively. The AFM images demonstrate that the structure of the beta-carotene is spherical, and the average diameter of this molecule is 37.22 nm. The findings of the analysis show that the extraction and isolation of β-carotene from the carrot extract was achieved.

Keywords

Green Synthesis, Nano β-Carotene, Medicine Transport Systems, Biomedical Applications, Controlled Drug Release, Pharmaceutical Carriers

Cover Page Footnote

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RESEARCH ARTICLE

A Convenient Green Method to Synthesize β-Carotene from Edible Carrot and Nanoparticle Formation

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Abstract

A simple green chemistry approach was used to synthesize β-carotene from edible carrot without using solvent and heat. In this study, beta-carotene was extracted from edible carrots through a natural extraction method with several physical technical steps. The extracted sample was isolated using high-performance liquid chromatography (HPLC). A β-carotene peak was detected from HPLC with an absorbance maximum (2.135 mAU). The absorption of UV−Visible spectrum indicated that beta-carotene absorbs most strongly between 230-300 and 400−500 nm. The functional groups, namely, C═C (1649 cm^{-1}), ═C−H (900-900 cm^{-1}) vibrations of antisymmetric deformation of CH_3 groups and CH_2 groups (1350-1450 cm^{-1}) of beta carotene were identified by using the FTIR test. The 537, 538, and 539 peaks for all carbon atoms (C-12), one of the 40 carbon atoms is (C-13) and two carbon atoms as (C-13) were indicated using FTMS. The resonance relates to the protons connected to methyl and methylene groups and those attributed to the vinylic protons (H−C═C) were observed between 1-2 and 5−6 ppm, respectively. The AFM images demonstrate that the structure of the beta-carotene is spherical, and the average diameter of this molecule is 37.22 nm. The findings of the analysis show that the extraction and isolation of β-carotene from the carrot extract was achieved.

Keywords: β-Carotene, Carrot, Nanocarriers, Controlled drug release

1. Introduction

In the recent century, the applications of renewable resources turn out to be more essential for human daily life because they are friendly to nature [1]. The applications and development of green chemistry in academia and industry have been discussed by researchers in the literature [2−5]. The study of nano-sciences especially natural nanomaterials has received increasing attention from many scientific researchers for the progressing trend of nanomaterial applications [6−8]. Generally, edible carrots include the valuable type of carotenoids which are fat-soluble compounds [9]. Carotenoids are colored red-orange pigment that has large, conjugated hydrocarbon skeletons [10]. The basis for the special properties of carotenoids is the conjugated double bond system, which makes them easy to interact with other material due to their delocalized π-electrons having high energy and needing slight energy for excitation [11]. β-carotene is considered a major of carotenoids (Fig. 1), which is known to show provitamin A activity [10,12]. In addition, β-carotene has antioxidative properties [13,14], therefore, they are expected to reduce the risk of different diseases [15,16]. The consumption of these compounds in different fruits and vegetables ultimately has led to decreased risk to develop such as cancer disease [17−20], cardiovascular diseases [21,22], and those age-related ones [23−25]. In contrast, there are several studies in recent years were found to be contradictory [26,27] and some of these show a negative correlation between β-carotene and some diseases, for example, lung cancer and cardiovascular disease for individuals with smoking activity [28,29].

The possibility of preparing organic molecules using the basis of biomass has previously been investigated. For example, beta-carotene molecules were prepared from the apricot plant as water-

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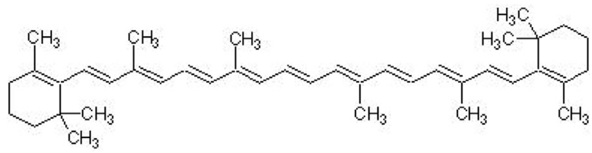

Fig. 1. Molecular structure of beta-carotene [30].

soluble particles using the technique of separating the aqueous phase from the organic layer and diagnosing them as nanoscale particles [31]. The nanoparticles prepared in this way were distinguished by high thermal stability, with nanoscale sizes ranging between (15–250), which were coated with gelatin to be applied in the food and pharmaceutical industry [32]. The effect of nano-encapsulated using β-carotene was investigated by several researchers [33,34]. Recent studies have investigated the possibility of applying nanoencapsulation using beta-carotene molecules in the food sector [35,36]. In addition, the nanoparticles with drugs such as aspirin and metronidazole were applied to verify their ability to nanoencapsulation with high efficiency [37]. The different extraction methods using various types of supplements were utilized and confirmed depending upon the levels of β carotene [38]. This study aims to prepare the nanoparticles of β-carotene from edible carrots by using a convenient green method to prove their ability to nanoencapsulation with high efficiency in part II of the study. Interestingly, there is no study has been previously reported to prepare β-carotene from Iraqi carrots. This synthesizes is important to recognize the nutritional and therapeutic significance of beta carotene.

2. Materials and methods

2.1. β-carotene extraction

The used carrots were obtained from the local supermarket in Karbala, Iraq. The samples were washed using deionized water and cut into tiny pieces to obtain a homogenous sample. The extracted juice was prepared by using a juice extractor without using solvent and heat which may affect the chemical structure of the extracted material or lose the protons that are their presence has a role in the required physical and chemical adsorption and interactions. The extracted sample was centrifuged (15 min) at 7500 rpm and filtered using a piece of cloth. From 100 g of sample, 10 g were selected and kept at −4 °C temperature for extraction. The prepared sample (10 g) was homogenized in 30 ml of acetone. The product solution was filtered using Buchner's funnel, then acetone was used to wash the residue until become colorless. The filtrate was mixed with 20 gm of anhydrous sodium sulfate while the residue was discarded. Then, anhydrous sodium sulfate was separated through filtration and a rotatory evaporator was used to reduce the volume of extract. The extract solution was moved into a volumetric flask (100 ml) and filled with enough acetone and water; therefore, the final extract contains 80% of acetone [36]. Samples of beta carotene (20 μl) were injected into the HPLC system installed at the Laboratory of Chemistry Department, Kerbala University. The chromatographic conditions were Shimadzu HPLC program comprising LC-1000 pump having C18 column connected with LC 250 UV/VIS detector. This instrument was calibrated by using mobile phase (Acetonitrile, dichloromethane, and methanol by the ratio of 70:20:10, respectively) at the rate of 2 ml per minute. Wavelength has been selected at 450 nm, and the column pressure was kept at 1800–2000 PSI.

2.2. Instrumentations

The instruments used in this study were HPLC systems (Shimadzu) containing LC-, and C18 columns connected with UV/Vis detector; Fourier-transform infrared spectroscopy (FTIR) (Shimadzu-8000); Fourier Transform Mass Spectrometry (FTMS); Ultraviolet–Visible (UV–Vis) (Shimadzu) spectrometry, a Bruker 300 MHz NMR Spectrometry and atomic force microscopy (AFM) through Scanning Probe Microscopy (SPM) technique (AA3000). The analyses of FTMS and 1H NMR were carried out in Australia at the University of New South Wales.

3. Results and discussion

3.1. Separation method

The extract carrot juice was analyzed using HPLC to determine the content of beta carotene in this juice [39]. The results show that the 100 gm of carrot includes thousands of micrograms of beta carotene (11,620 μg/100 g). The findings in this work are approximately in agreement with those described by other studies in which β-carotene quantity in carrot was 11,210 μg/100 g. This variation in beta carotene content is probably due to the influence of several factors such as varietals difference, diameter, extraction methods, column materials, experimental conditions, and used mobile phase [36]. Interestingly, the parameters such as temperature during the analytical process, extraction process of the samples, and the storage time of carrot must be

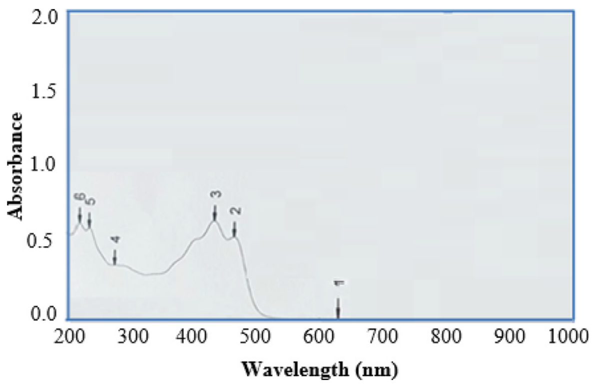

Fig. 2. UV–Visible spectrophotometry of beta-carotene.

kept in control because they have a tremendous effect on the results [40,41]. A chromatogram chart shows that the maximum peak of β-carotene was identified between 2 and 2.5 min. In addition, there are lower peaks were observed at various wavelengths that may be returned to impurities [42]. The level of purity was determined by measuring the melting point of extracted beta carotene, then compared with the value of pure material. The value of melting point for extracted beta carotene (170 °C) was lower than pure material (180 °C). This is due to the extracted material including soluble impurities, which are dissolved at lower melting points when compared with pure substance [43].

3.2. UV–visible analysis

Analysis of UV–visible spectrum for beta-carotene is presented in Fig. 2. The instrument conditions (wavelength range: 190–1100 nm, scan mode: single, scan speed: fast, sampling interval: 1, slid width: 1 nm, S/R exchange: normal) have been used in this analysis. An electronic transition was observed at 210–230 and 400–500 which are revealed to $\pi \rightarrow \pi^*$ transitions because of the electronic conjugation system at the axis of a molecule by double (C=C) bonds [44]. The main reason is that the energy gap between Homo and Lumo for conjugate double bonds is less than the energy difference for an isolated double bond. In addition, the λ_{max} increases when the number of conjugated double-bound increases [45].

3.3. FTIR analysis

The dried powder of β-carotene obtained through extracted juice was subjected to FTIR spectroscopy measurements using Shimadzu- 8000 instruments. The sample was combined with KBr, in the sample: KBr ratio of 1:10, then crushed into powder and compressed in a thin layer by a tablet machine at 1 cm^{-1} resolution within the frequency ranges between 4000 and 400 cm^{-1} [37,39]. Beta-carotene is considered as asymmetric conjugated polyenes, so two bands for (C=C) stretching have appeared, the first around 1649 cm^{-1} assigned to (C=C) that clear from (CH_3), while the second at 1556 cm^{-1} for (C=C) bonded with (CH_3) [46,47]. The band at 1456 cm^{-1} is due to scissoring (CH_2) bending, while bands around 1421 cm^{-1} and 1367 cm^{-1} are attributed to asymmetrical and symmetrical (CH_3) bending [48–51]. The oscillations of the –OH groups of water were found between 3000 and 3600 cm^{-1} as a broad peak [52]. The results of the FTIR spectroscopy study revealed that β-carotene is present in the carrot, as shown in Fig. 3.

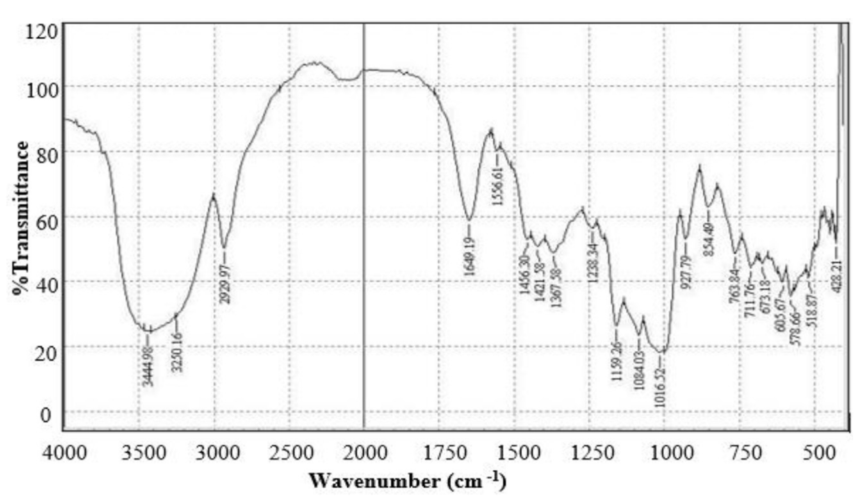

Fig. 3. FTIR for the beta-carotene molecule.

3.4. FTMS analysis

Fourier Transform Mass Spectrometry (FTMS *m/z* + p NSI Full ms [100.00–2000.00]; RT:0.02–0.52AV:10NL:2.26E7; AV: 10 T). In this technique, the cyclotron frequency of ions in a fixed magnetic field is used to determine the *m/z*. The system provides the highest mass resolution achievable of all mass spectrometers and can provide mass resolutions of 1,000,000 [52–54]. Mass spectroscopy analyzes demonstrated the peaks of β-carotene, as shown in Fig. 4. The beta-carotene nanoparticles have taken the following molar masses 537.44548, 538.44888, 539.45228, respectively, which are simply corresponded to the molecular ion with a proton added, which is a function of the ionization method used. The 537.44,548 peak is with all carbon atoms are C-12, 538 is with one of the 40 carbon atoms is C-13 (since C-13 is about 1.1% of natural carbon, and the peak at 539 has two carbon atoms as C-13. The resulting compound and molar mass (537.44548 g/mol) belong to the molecular formula ($C_{40}H_{56}$ + H), which are formulas with charges in which the proton exchanges by gaining or losing on the surface of the molecule structure which belonging to the beta minutes.

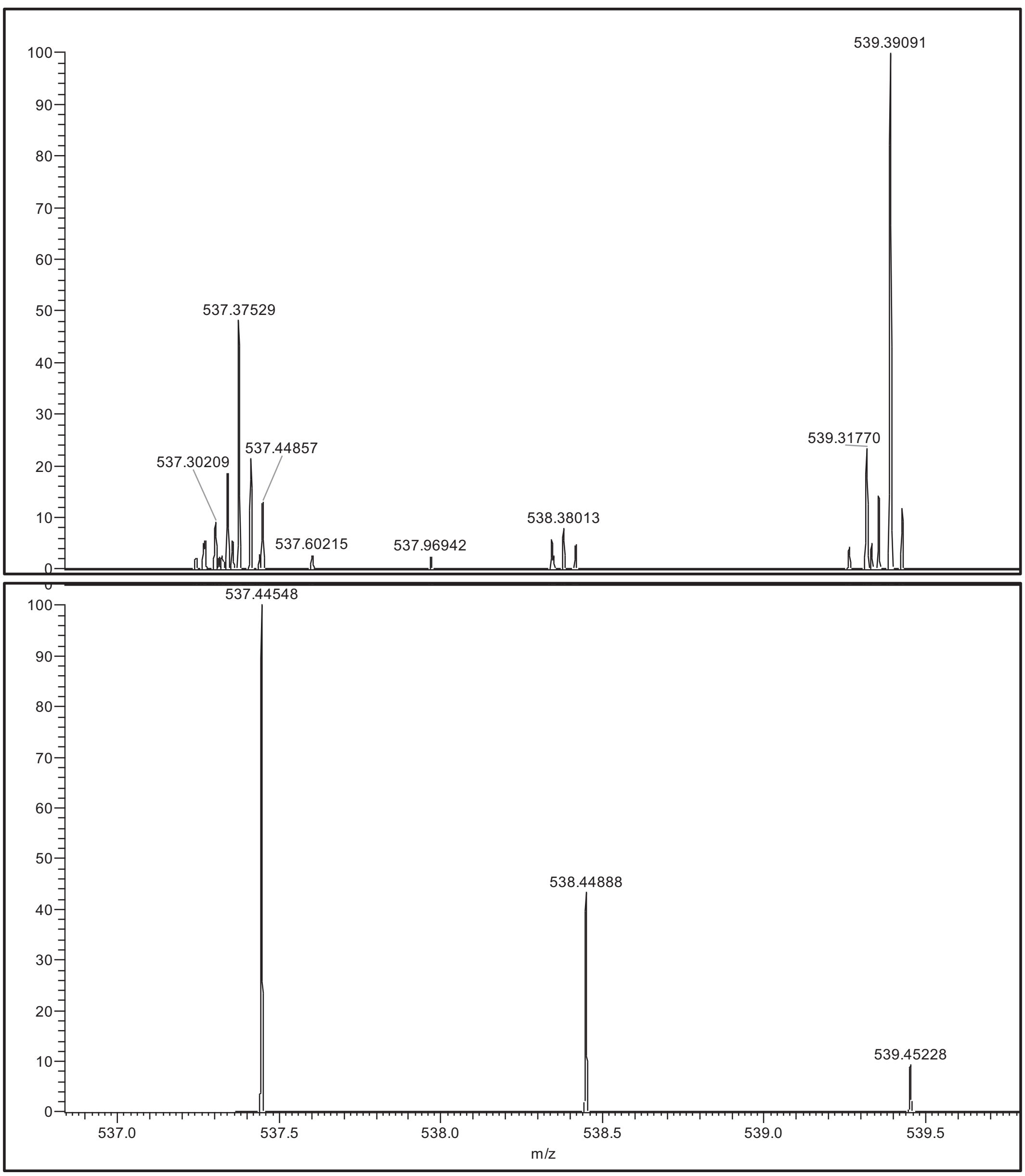

Fig. 4. FTMS spectrum of prepared particles of beta-carotene.

3.5. 1HNMR spectra

1HNMR was measured in a Bruker 300 MHz instrument. The 1HNMR spectra of beta carotene (BC) and carrot extract (CE) are shown in Fig. 5. In comparison between the two spectra, the broad multiple peaks (between 3 and 4 ppm) are found in CE which suggest the presence of a mixture of saccharides [42], while these peaks are disappeared in BC. The resonance at 5 and 6 ppm is attributed to the protons on a double bond, whereas the resonance of protons attached to methyl is observed between 1 and 2 ppm [54]. In addition, the 1HNMR spectra of BC showed the resonances that correspond to the protons attached to the ring were observed between 1.5 and 2.5 ppm, but the aromatic protons are found at 8.2 ppm in CE and disappeared

Table 1. The properties of surface topology and nanoscale for beta carotene from AFM studies.

Parameter	Beta-Carotene
Core roughness depth (nm)	17.60
Roughness average (nm)	6.89
Surface area ratio	10.9
Average diameter (nm)	37.23
Average height (nm)	27.87

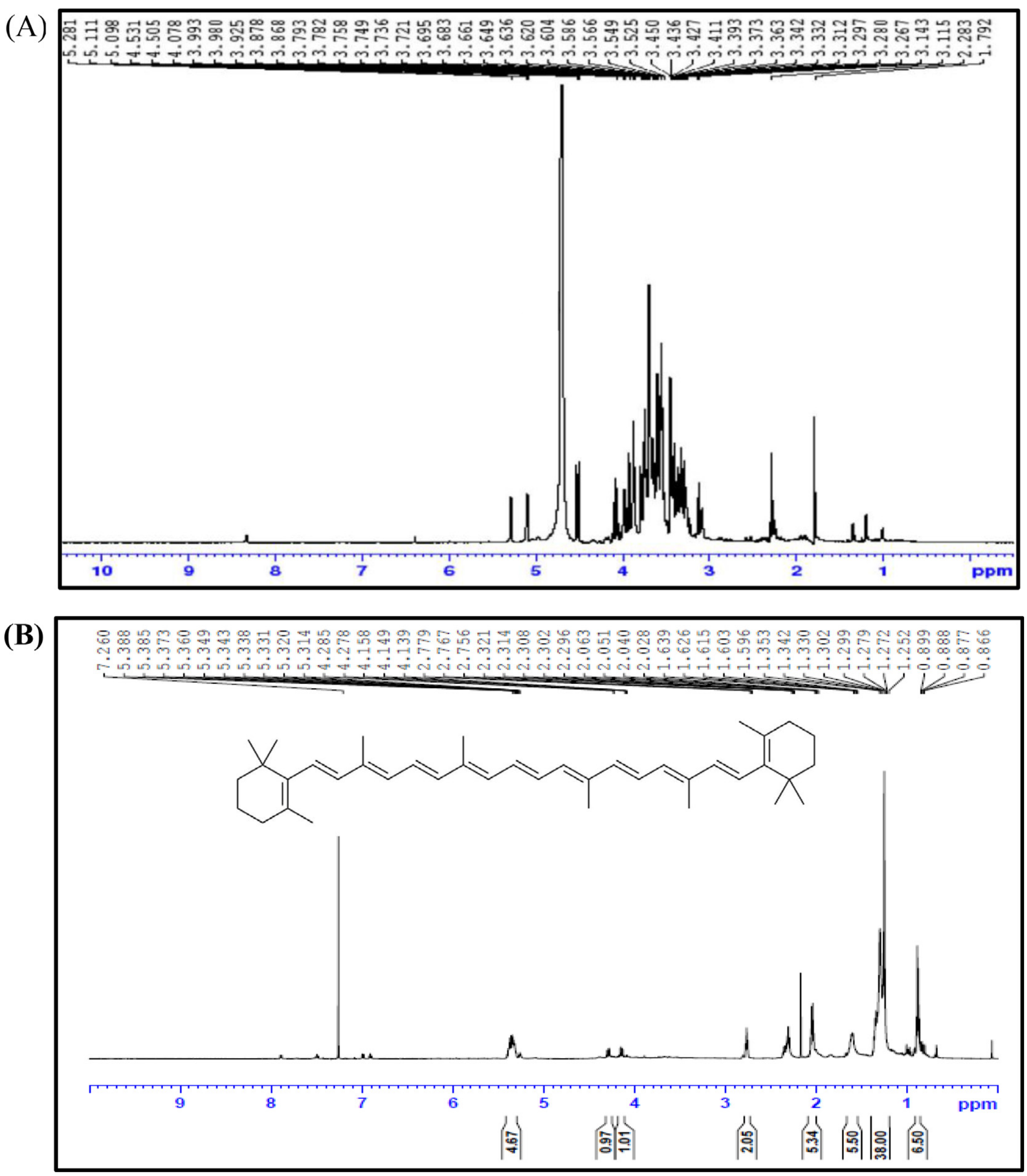

Fig. 5. 1H NMR spectrum of (A) carrot extract [42] and (B) Beta Carotene in $CDCl_3$ at 600 MHZ.

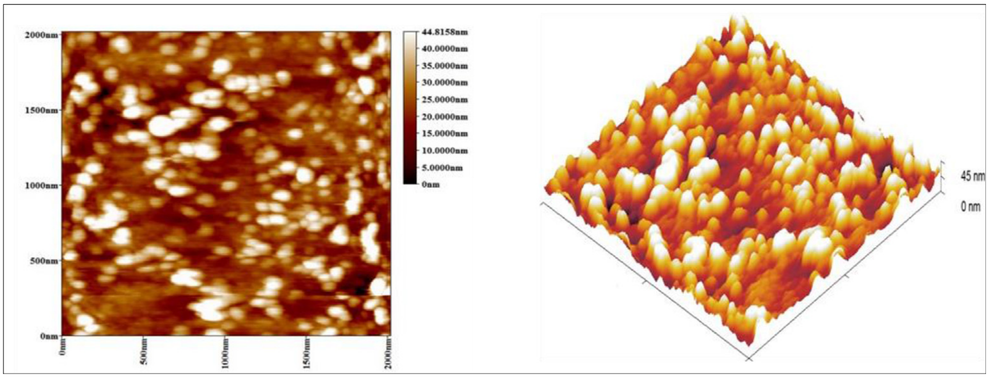

Fig. 6. AFM images of Beta-Carotene.

in BC [42]. In conclusion, the FTMS, 1HNMR, IR, and UV/visible spectra confirmed that the isolated material was beta-carotene.

3.6. AFM analysis

The atomic force microscopy (AFM) is a dynamically evolving field and provides new imaging types to make the technique even more useful, faster, less damaging, and quantitative for polymer science applications [55]. AFM/SPM mapping was performed to evaluate the topography, surface potential distribution, and nanoscale properties for the beta-carotene molecules [56]. Table 1 and Fig. 6 show the values of core roughness depth (17.60 nm), surface roughness average (6.89 nm), and surface area ratio (10.9), along with a smaller average of diameter (37.23 nm) and height (27.87 nm). These properties have many useful advantages, therefore can be used in different vital aspects such as medicine and industrial applications such as pharmaceutical carriers.

4. Conclusions

In this work, natural extraction along with several physical technical steps was used to prepare a beta carotene by using an edible carrot. The extracted beta carotene has been characterized using HPLC, UV−Vis, FTIR, FTMS, and 1H NMR spectroscopy. These techniques confirmed that the isolated material was beta-carotene. The surface and nanoscale properties were investigated using AFM. The properties are confirmed that beta-carotene has many useful advantages, which can use in different medicine and industrial applications.

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