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New Method for Encapsulation of Oregano Essential Oil into Carbon Nanotubes

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Abstract: In this paper it is proposed a new method of encapsulation of oregano essential oil (EO) into carbon nanotubes, singlewalled (SWCNTs) and multiwalled (MWCNTs). Multiwalled carbon nanotubes were functionalized by oxidation methods to obtain carboxilated carbon nanotubes MWCNT-COOH and aminated carbon nanotubes MWCNT-NH_2. The effect of encapsulating matrix on protection and delivery of oregano essential oil was studied. We will refer to preparation by encapsulation of carbon nanotubes with oregano essential oil in order to improve characteristics that can be used in biomedical applications. EO was encapsulated in carbon nanotubes by physical immersion and the samples were ultrasonated for 2 hours at 37ºC. After the treatment of carbon nanotubes with oregano essential oil, these varieties of materials were inserted into a collagen gel and lyophilized in order to obtain collagen matrices. The structure of the new functionalized carbon nanotubes immersed in a collagen matrix was identified using infrared spectroscopy (FTIR analysis) and morphological features were studied by transmission electron microscopy (TEM) and scanning electron microscopy (SEM).

Keywords: carbon nanotubes, oregano oil, TEM, FTIR.

Introduction

Oregano is an aromatic plant from Mediterranean countries widely used as food ingredient due to its specific flavour. Moreover, oregano oil is a strong antioxidant [1] with very good antimicrobial properties being a good alternative for food preservative. The use of EO as health promoting substance is directly related with some components of oregano such as carvacrol and thymol.

Microencapsulation is the technique that allows sensitive ingredients to be entrapped in every kind of homogenous or heterogeneous matrix but the choice of material is an important key for the success of encapsulation process.

Carbon nanotubes (CNTs) [2,3] were widely used because posses exceptional electrical, thermal and mechanical properties and is not being degraded in human body; they can be used to deliver small organic drug molecules into the diseased cells thus preventing normal tissue damages. Due to the chemical oxidation CNTs can be very easy functionalized and they can be used as targeting materials in combination with cisplatin (potent anticancer agent). Single wall carbon nanotubes (SWCNTs) potential can be exploited by uploading the form of anti-neoplastic compound shell at the external tube by a covalent bond. The drug was then released as active form as the reduction reaction in acidic endosomes and lysosomes.

2.Experimental methods

2.1. Materials and Reagents

High-purity SWCNTs were purchased from Sigma Aldrich. These SWCNTs were produced by a chemical vapor deposition process yielding particle external diameters of less than 2 nm with lengths ranging from 0.5 to 40 microns and a purity > 90%.

Multiwall Carbon Nanotubes (MWCNTs) were purchased from Sigma Aldrich having more than 90% carbon basis and D x L 10-15 nm x 0.1-10 µm, produced by Catalytic Chemical Vapor Deposition (CCVD). Oxidation was made using a mixture of 98% sulfuric acid (Merck).

2.2. Equipment

FTIR spectra of functionalized MWCNTs were registered on a Perkin Elmer, Spectrum 100 equipment in 400÷4500 cm^{-1} range with 4 cm^{-1} resolution and 32 scans.

Nano-sized particles were investigated using TEM analysis with a microscope Philips EM-410, 60kV and by SEM using a SU8230 microscope.

2.3 Procedure

Multiwalled carbon nanotubes were functionalized with carboxil –COOH, cisplatin -CDDP and amino $-NH_2$ groups.

Functionalization of MWCNTs with –COOH groups:

MWCNTs (2.0 g) were dispersed in 98% concentrated sulphuric acid under ultrasonication at 50°C for 6 h to produce oxidized carbon nanotubes (MWCNT-COOH) [4]. The samples were washed with ultrapure water and dried at 50 °C for 12 h.

Obtaining of MWCNTs with –CDDP

CDDP (5 mg) was added to MWCNT-COOH solutions in 1 ml saline solution. They were ultrasonicated for 48 hours at 50° C and filtered.

Functionalization of MWCNT-NH_2

Dried MWCNT-COOH (0.1 mg) was reacted with excess $SOCl_2$ (25 mL) at room temperature for 30 minutes. The residual $SOCl_2$ was removed by washing with tetrahydrofuran THF and filtered with ultrapure water.

The MWCNTs were dried for 20 minutes at the room temperature.

The new functionalized nanotubes MWCNT-$SOCl_2$ (10 mg) are added in etilendiamine in excess for 10 hours at the room temperature. The mixture was washed with THF and filtered. The nanotubes were dried at 80°C /10 hours [5].

Encapsulation of oregano essential oil

The encapsulation of oregano essential oil consists of adding 5 ml of EO into samples that contain carbon nanotubes (functionalized and nonfunctionalized, single or multiwall), under ultrasonication for 2 hours at 37ºC.

We will refer to 5 types of samples such as:

-sample 1: SWCNTs+EO;
-sample 2: MWCNTs+EO;
-sample 3: MWCNT-COOH+EO;
-sample 4: MWCNT-CDDP+EO;
-sample 5: MWCNT-NH_2+EO.

After that encapsulation the samples was characterized by TEM and FTIR.

Type I collagen of bovine origin was extracted by the currently used technology as previously described [6]. The collagen (Coll) was obtained as gel in native form with fibrillary structure with an initial concentration of 2.11%, pH 2.5 and free of fat and ash. Glutaraldehyde (GA) was supplied by Sigma-Aldrich (Germany) and sodium hydroxide from Merck (Germany). All the chemicals used in this work were of analytical grade and the water was distilled. The obtained functionalized carbon nanotubes encapsulated with oregano essential were added to collagen gel with ratio 1:10 (reported to dry collagen). The composite gels based on collagen as such (reference sample) and collagen with CNTs were adjusted at physiological pH (7.2 – 7.4), 1.2% collagen (dry substance) and then cross - linked with 0.5% GA. The cross-linked gels were frozen at -40^{0}C for 12 hours and then freeze-dried according to the method previously described [7] using the Christ Model Delta 2–24 LSC freeze-dryer (Germany). 3D spongious composites were obtained and characterized as by SEM.

3.Results and discussions

3.1 TEM analysis:

Nanostructured composite morphologies are investigated using a transmission electron microscope EM-410, 60kV. The morphologies obtained for every sample are presented in figures 1.

For the first sample (fig. 1a) the morphology is specified for single-wall carbon nanotubes. The structure is uniform and tubular distributed on the surface of a cooper grid. The average diameter of SWCNTs nanotubes was measured with ImageJ program and the value is almost 1.2±0.2 nm. From place to place, it is visible on the TEM micrographies, the encapsulation of oregano oil into SWCNTs.

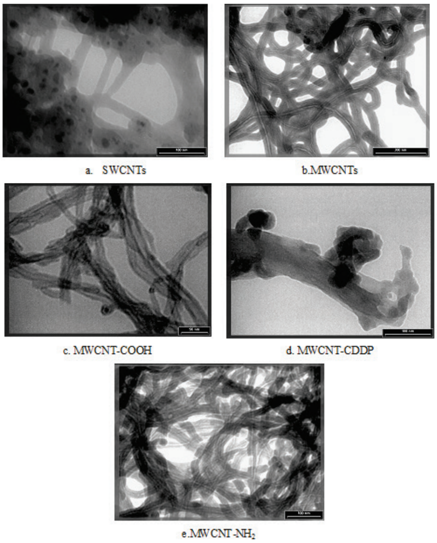

a. SWCNTs
b.MWCNTs
c. MWCNT-COOH
d. MWCNT-CDDP
e.MWCNT-NH$_2$

Figure 1. TEM micrographies for samples encapsulated with oregano essential oil

The encapsulation of EO into MWCNTs is put in evidence in fig. 1b. The net of nonfunctionalized multiwalled carbon nanotubes is very dense and encapsulation takes place at the ends of carbon nanotubes, at the extremities. The average diameter of these kinds of carbon nanotubes was also measured with ImageJ soft and the value is almost 9±1 nm. The encapsulation of oregano essential oil it is also very well put in evidence in images 1c, d and e.

3.2 FTIR determinations:

The samples were analyzed using FTIR and infrared Microscopy Spectral data were recorded by an ATR Perkin-Elmer equipment. In figures 2 are presented the FTIR of super reposed of individual spectra of 1÷5 samples.

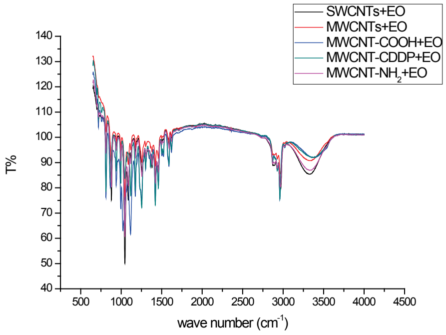


Figure 2. FTIR for samples encapsulated with oregano essential oil

The –COOH and –NH$_2$ functionalization is put in evidence by FTIR measurements.

FTIR has been used to map the chemical distribution of carbon nanotubes SWCNTs, MWCNTs, acid treated MWCNT-COOH, MWCNT-CDDP and MWCNT-NH$_2$.

FTIR spectra for the super reposed of 1÷5 samples show a broad peak at 3406 cm^{-1}, which refers to the O-H stretch of the hydroxyl group which can be ascribed to the oscillation of carboxyl groups.

Carboxyl groups on the surfaces of MWCNTs could be due to the partial oxidation of the surfaces of MWCNTs during purification by the manufacturer, 1626 cm^{-1} and is associated with the stretch mode of carboxylic groups (O=C−OH and C−OH), as observed in the IR spectrum of MWCNT-COOH.

Carboxylic groups are formed due to the oxidation of carbon atoms on the surfaces of the MWCNTs by sulphuric acid. The peak at 1878 cm^{-1} can be associated with the O−H stretch from strongly hydrogen-bonded −COOH. The peak at 1592 cm^{-1} is related to the carboxylate anion stretch mode. The peak at 1629 cm^{-1} can be associated with the stretching of the carbon nanotube backbone. The peaks at around 2870 and 2927 cm^{-1} correspond to the H−C stretch modes of H−C=O in the carboxyl group.

For MWCNT-CDDP present a peak at around 868 cm^{-1} specific for –CDDP.

For MWCNT-NH_2 the bands at 2929 and 2873 cm^{-1} represent asymmetrical and symmetrical stretching of CH_2 groups. The C–N stretching vibration and the scissoring in-plane N–H distortion of free primary amine group are observed at 1047 cm^{-1} and 1622 cm^{-1}, respectively. The broad band at 3355 cm^{-1} is attributed to the NH_2 stretching.

We supposed that any other peaks presented in individual spectrum of each sample are specific for oregano essential oil encapsulation.

The addition of EO resulted in a markedly increased in intensity of the CH stretching peak at 2867–2955 cm^{-1}, indicating an increase in the content of ester groups, which might come from EO molecules. The peak at 2961 cm^{-1} is due to the presence of –CH stretching, the peak at 1594 cm^{-1} represent the N-H bending, at 1459 cm^{-1} is CH_2 bending, at 1255 cm^{-1} and 1121 cm^{-1} is put in evidence the –C-O-C- stretching and 936 cm^{-1} is specific for C-H bending.

Figure 3 present some literature results for FTIR spectra [8].

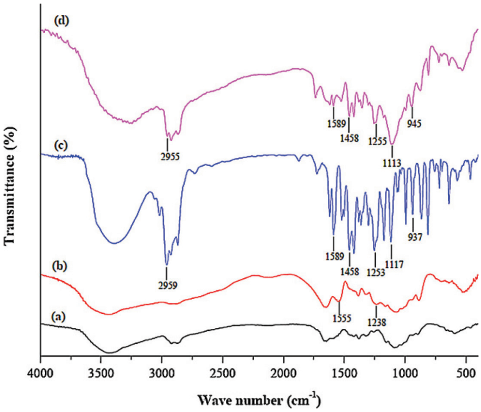


Figure 3. Literature: (c) spectrum is for oregano oil [8] and (d) is for oregano oil+chitosan - seems similar with ours spectra

3.3. SEM morphology

The morphological aspects of the samples surface, with CNTs and EO into the collagen matrix and the pore interconnectivity were determined by Scanning Electron Microscopy, using a Hitachi SU8230 microscope at 5.0 kV

The morphology is presented in figure 4.

The SEM investigation offer information about the morphology and distribution of the particle to/at the collagen matrix.

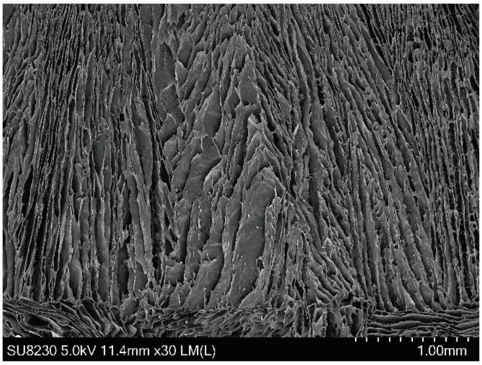
In figure 4a is presented the morphology of the composite that involves SWCNTs, oregano oil and collagen.

The figure 4b presents the morphology of the composite made by MWCNTs, oregano oil and collagen.

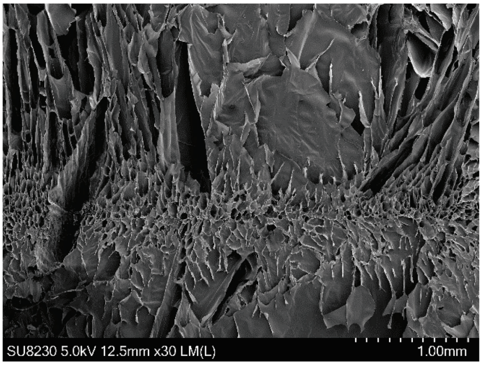
Both pictures show some similarities. The structure is a porous one.

The morphological results by SEM analysis revealed a differentiated homogeneity and pore interconnectivity in our composites.

The walls of the collagen network appear to be thicker for the composite containing single-wall carbon nanotubes than for the composite that contain multiwall carbon nanotubes.



a)



b)

Figure 4. SEM image of the collagen based matrix: a) with SWCNTs; b) with MWCNTs

4.Conclusions

Results show that both kind of carbon nanotubes, single or multiwalled were encapsulating quite well the oregano essential oil. The SEM images of the composite revealed a high porous structure, common in all samples. This kinds of materials encapsulated further in a collagen matrix seems to be very promising for biomedical applications. More tests will be proceed in order to study the biocompatibility of our composites based on collagen matrix.

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