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Synthesis, Characterization and Ibuprofen Release Profile of Alumina Mesoporous/CNT Nanocomposite

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Alumina/CNT porous composites were synthesized using isopropoxide aluminum as an alumina source, multi-wall carbon nanotube as reinforcement and Pluronic F127 as a polymeric template by sol gel method. Pores were produced after calcinations at 700˚C. One of the considered applications of these nanocomposites is its used as a drug delivery system (DDS); so, ibuprofen is used as a model drug for investigating this application. In order to improve chemical interactions between drug and its career, surfaces of nanocomposite were modified using post-synthesis method by 3-aminopropyltriethoxysilane (APTES). To investigate cytotoxic effect of nanocomposite and clinical applicability, cell culture test (MTT assay) was used. Drug release tests were done in Phosphate Buffer Saline (PBS). Physical and chemical properties of the synthesized materials were characterized by X-Ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Energy Dispersive Spectroscopy (EDS) and field emission scanning electron microscopy. Tests of drug loading and release were done by UV-Vis spectroscopy. The results of cell culture tests confirmed non-cytotoxicity of synthesized nanocomposite. It was found that, with increasing content of carbon nanotubes in composite and functionalizing the particle surfaces, drug loading was increased. Also, drug release of functionalized composites was slower than that of the non-functionalized material.

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1. Introduction

It is well known that high efficiency of mesoporous materials such as physical adsorbents is because of their high pore volumes and large specific surface areas. Designing and manufacturing porous ceramic oxide systems in various applications, especially in managed therapeutic methods, by drugs are very useful. Recently, mesoporous materials have attracted special attention in this field. In fact, high pore volume of mesoporous materials allows biologically active molecules with different sizes to be placed in their pores [1, 2, and 3]. Since absorption of molecules in mesoporous materials is a surface-dependent phenomenon, high surface area of these materials results in greater absorption of biologically active molecules. So, synthesis of mesoporous materials, especially-ordered mesoporous materials, is very essential. It has been shown that the most effective way for controlling release profile of a drug from mesoporous materials is to optimize drug–surface interaction via suitable surface chemical functionalization. For example,

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when alumina mesoporous materials are functionalized by amino groups, the ionic interaction between carboxyl groups on ibuprofen and amino groups on matrix leads to a slower release of drug in comparison with the rate of release from the non-functionalized material with surface hydroxyl groups [4, 5].

Most methods of producing these composites are based on dissolving and mixing raw materials in liquid phase and then heat treatment of products at high temperatures like calcinations method. Nowadays, various methods are applied for synthesis of alumina-carbon nanotube composite powders, which include colloidal process, Mechanical Alloying (MA) or mechanochemical, in situ methods in which carbon nanotubes grow on alumina substrate containing metal catalyst particles such as iron, nickel, cobalt and electrophoretic deposition (EPD) and sol gel method [6-8].Sol-gel method, because of its ability of producing nanoparticles with uniform size and shape and easy control of reaction parameters has attracted great attention. A majority of the published literature on mesoporous oxide DDS is based on silica with pores of single size ~2.5 nm. In this research, the main focus is on synthesis and evaluation of structural properties of mesoporous alumina carbon nanotube composite powders for medical applications.

Until now, no thorough research has been done to investigate the use of porous alumina nanocomposites with carbon nanotubes as drug carriers; therefore, the aim of this study is to investigate the possibility of synthesizing porous alumina nanocomposites containing carbon nanotubes and the possibility of its use as a drug carrier. The considered application of the nanocomposite is loading and release of ibuprofen and, after ensuring their non-toxic effect, the related tests are performed.

2. Experimental Procedure

2.1. Synthesis of Alumina Mesoporous Powder

To begin the process of synthesizing porous alumina, the method used by Stacy M. Grant et al. was applied [9]. Synthesis details were as follows: approximately 9 g F127 was poured into 240 ml ethanol under stirring conditions for about 1 h at room temperature to form a completely homogeneous solution. Then, 12.24 g of aluminum isopropoxide powder with 40 ml ethanol for complete transfer was added to the above solution. Nine ml nitric acid was added drop wise to the solution as the reaction catalyst and the media temperature was increased to about 45° C. The new solution was treated under vigorous stirring at this temperature for 5 h to complete the hydrolysis reactions. Then, the resulting solution was aged over night at room temperature and drying was done by simultaneous stirring and heating at 60-65˚C. The final product of this process was a porous material and, after partial crushing, it was heated in a furnace for 2 h at 700° C at heat rate of 1°C to remove surfactant; thus, approximately 3 g of mesoporous alumina powder was synthesized.

2.2. Synthesis of Alumina Mesoporous/CNT Powder

The first step to start synthesis procedure was to prepare carbon nanotubes for better use in the solution [3]. In order to enhance hydrophilic property and prevent agglomeration problems, CNTs were treated in mixture of nitric acid and sulfuric acid (ratio of 3:1) at 85°C for 3 h under slow stirring and 15 min sonication to form functionalized group on surfaces of CNTs. Then, this solution was alternately washed with distilled water using a centrifuge until reaching neutral pH and was dried at approximately 70˚C over night.

To add carbon nanotubes into alumina solution, proper amounts (1, 2, 3, 4 and 5 wt% of final product) of functionalized powdery CNT were poured into the solution of ethanol and distilled water (ratio of 2:1) for 10 min in the ultrasonic bath. Two hours after adding aluminum isopropoxide and nitric acid to the previous solution, the black solution containing nanotubes was added to the medium and placed for 15 min in the ultrasonic bath. The rest of synthesis procedure was followed as before.

2.3. Functionalization of Nanoparticles

After synthesis of MPA/CNTs, the composites were functionalized by 3-amino propyl tri methoxy silane (APTES) to form functional groups on particle surfaces.One ml APTES was added to 50 ml ethanol and the solution was stirred slowly at room temperature for 1 h. One g of the powder composite was added to the solution and stirring was continued for another 6 h. After formation of functional groups on the surface of particles, the solution was removed by a paper filter and the powder was washed three times with ethanol to

eliminate excess APTES. After the washing process, the final powder was dried for 24 h at room temperature and other 24 h at 60˚C.

2.4. Cell culture Test and Cytotoxicity Evaluation

The human osteosarcoma cell lines (MG-63) were obtained from National cell bank of Iran and cultured in Dulbecco's Modified Eagle's Medium (DMEM, GIBCO, Scotland) supplemented with 10% fetal calf serum (FCS) (NanoBioArray, Iran), 100 u/ml penicillin and 100 µg/ml streptomycin (Sigma, USA).

A number of 1×10^4 cells per 100 µl for each were well seeded in a 96 well plate and incubated at 37°C under a humidified atmosphere with 5% CO_2. After 24 hours, 100 µl of each sample (200 µg/ml in culture medium) was added and incubation proceeded for the next 24 hours. Subsequently, the cell medium was discarded and replaced by 100 µl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma, USA) solution (0.5 mg/ml in phosphate buffer saline) followed by incubation for 5 hours at 37°C. The purple crystals were dissolved by addition of 100 µl of isopropanol (Merck, Germany) and incubation for 15 minutes. The absorbance of each was well measured by a microplate reader (STATFAX2100, USA) at 545 nm and normalized to control (culture medium without sample in the same condition). The viability was calculated according to the equation (1):

$$\text{Viability}\,\% = \frac{Sample\ Absorbance}{Control\ Absorbance} \times 100 \qquad (1)$$

Mesoporous alumina and carbon nanotubes have wide applications in biomaterial field; however, to date, no reports have been done on the use of composite of these two materials in medical applications. So, to ensure non-toxic effect of the composite and cell viability, cell culture test (MTT assay) is performed on the samples with 3 and 5 %wt of carbon nanotubes. The MTT assay is a colorimetric assay for measuring activity of cellular enzymes that reduces and breakdowns the tetrazolium yellowish crystals by the enzyme succinate dehydrogenase and forms insoluble blue crystals.

In this method, in contrast to other methods, cell picking and washing steps which often cause loss of some cells are eliminated and all the test steps are done in the same micro plate. So, this method has high repeatability, accuracy and sensitivity [10,11].

2.5. Ibuprofen Loading of Nanocomposite

Ibuprofen loading was performed in hexane solution at room temperature for 24 h. One thousand and two hundreds mg of composite was added to 10ml IBU–hexane solution, yielding a mixture of Al2O3 (in mg) to IBU–hexane solution (in ml) ratio of 120 mg L^{-1}.After 24 h, the powder was separated from the solution by centrifugation at 4500 rpm for 5 min and dried at room temperature for 48 h.

2.6. Ibuprofen Release

In vitro release of ibuprofen from nanocomposite was carried out by immersing the drug impregnated samples in PBS with pH of 7.4 at 37˚, with concentration of 2 mg of MPAC:IBU per ml of PBS. To prepare PBS solution, 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4 and 0.42 g KH_2PO_4 were added to 800 ml distilled water and pH was adjusted to 7.4 by adding HCl or NaOH solution. Then, volume was adjusted to 1 lit by adding distilled water. The amount of IBU release as a function of time at 37°C in PBS was determined by UV–Vis spectroscopy through monitoring changes in the absorbance at wavelength of 264 nm at suitable intervals.

2.7. Characterization of Mesoporous Alumina Composite Powders

Mesoporous structure of composites was determined by low angle X-Ray Diffraction (XRD) using BRUKER D8-Advance Cu-Kα X-ray beam with wavelength of 1.54 Å at voltage and current of 40 kv and 30 mA, respectively. In order to evaluate the morphology and particle size and also to identify nanostructure of the synthesized powders, field emission scanning electron microscopy (FESEM-Hitachi S-4160) was used. The influence of surface chemical groups on ibuprofen uptake and release were systematically studied using Fourier transform infrared spectroscopy and time-dependent UV–Vis spectroscopy.

3. Results and Discussion

Mesoporous oxides, with respect to their approximately amorphous structure, do not show common sharp peaks. For identifying their structures, researchers usually use low angle X-ray diffraction analysis with peaks before angle of 2°[12]. Accordingly, X-ray analysis is performed at 2Θ angles from 0.5 to 5° with step size of 0.01°.The presence of peak before angle of 2° is a structural

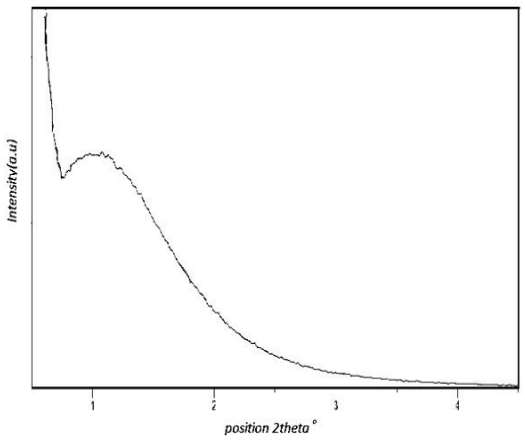

Figure1. Small angle X-ray diffraction pattern of alumina mesoporous

characteristic of nanometeric pore size of aluminastructural characteristic of nanometeric pore size of alumina nanoparticles, which is well observed in Fig.1 in the composite that is free from carbon nanotubes.

As shown in Figs. 2a and 2b, as the amount of carbon content increases, crystallinity decreases and weak peaks start to disappear so that the sample with 5 percent carbon nanotube peak was very weak and barely observed. This can be attributed to the presence of carbon nanotubes that interferes with self-assembly and exact ordering process of micelles in the solution phase; also, functionalized groups on surfaces of nanotubes have electrostatic fields which prevent ordering process of micelles. As the amount of nanotubes increases in the solutions, peaks' width increases somewhat which indicates reducing size of the nanopowders and more amorphous structure of pores.

3.1. FESEM

Fig. 3 shows microscopic images of synthetic MPA without CNTs. The particles had cauliflowermorphology and are spherical in shape with diameters of approximately 5-50nm. It is

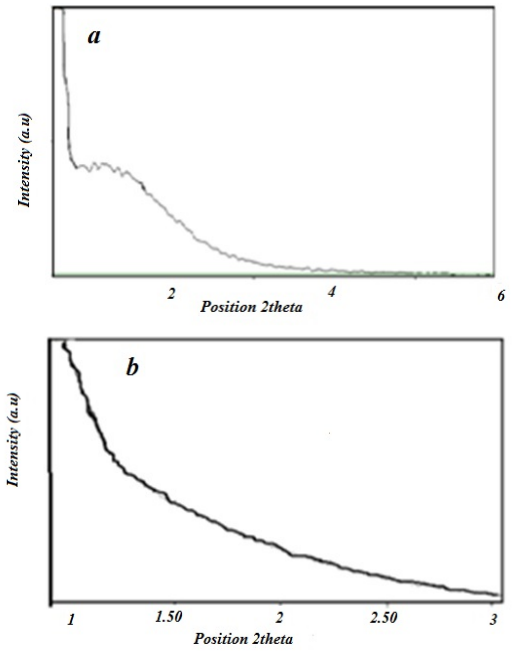

Figure 2. Small angle X-ray diffraction pattern of alumina mesoporous with a) 1% and b) 3% CNT

clearly shown that all of them exhibit well-defined 3D

connected networks containing nanosphere-like conglomerates, which are generated by removal of organic polymer during calcination in air. The micropores are occupied by the solvent during polymerization. After drying, the solvent is vaporized, leaving interconnected micropores.

Fig.4. shows SEM images of the MPA/CNT with varying amounts of CNTs calcinated at 700˚C. The alumina grain size gradually decreases with increasing CNT content. At 1-3 wt.% CNTs, grain size of the $Al_2O_3$is identical to pure alumina.

However, grain size of Al_2O_3 clearly decreases with increase in CNT content. Similar phenomenahave previously been reported forMWCNT/alumina mesoporous and MWNT/$BaTiO_3$ nanocomposites [13].

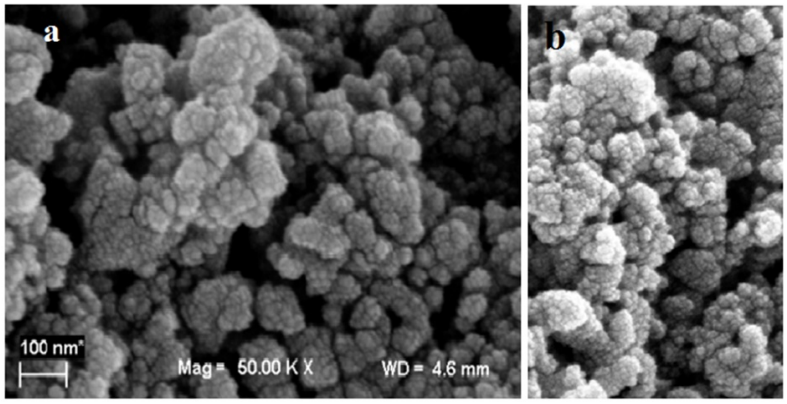

Figure. 3. SEM image of alumina mesoporous.

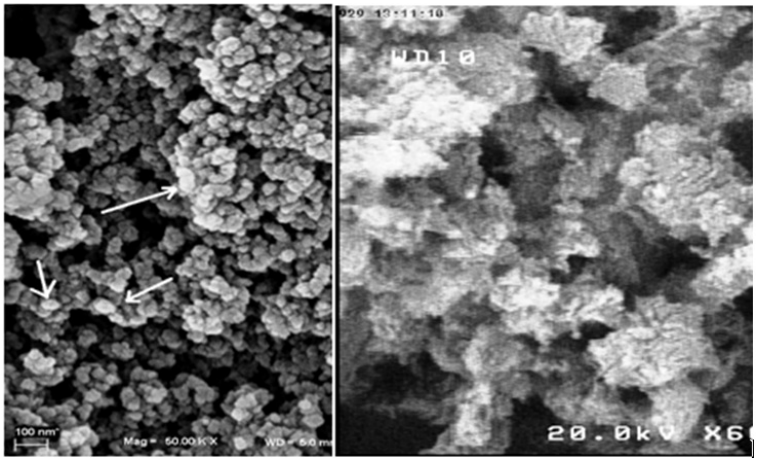

Figure 4. SEM image of MPA 3%CNT and MPA 5%CNT

The reduction of grain size is attributed to the pinning effect of CNTs, which restricts growth of the alumina grains and electrostatic fields around functionalized CNTs that prevents complete self-assembly mechanism of surfactant molecules [13].

3.2. EDX

EDX spectrum (Fig. 5) of the ordered mesostructured alumina displays strong peaks of Al and O elements without detecting other elements, except Cu and C deriving from residual template and carbon film coated copper grids, indicating successful formation of stoichiometric alumina with molar ratio of Al and O elements close to 2:3 with no further pollution. Peak of C detected in composites becames much stronger as the CNT content increases (Fig. 6).

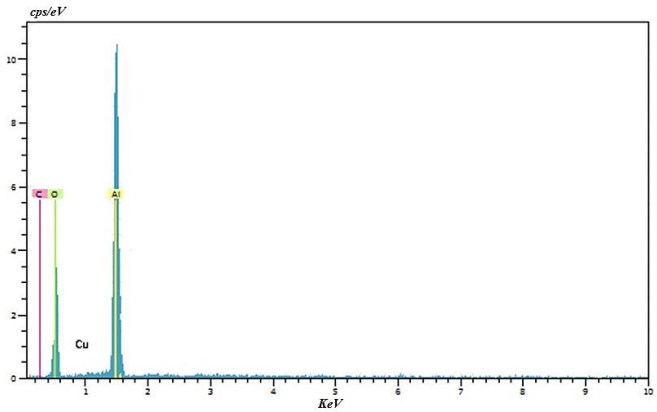

Figure 5. Energy dispersive X-ray of alumina mesoporous

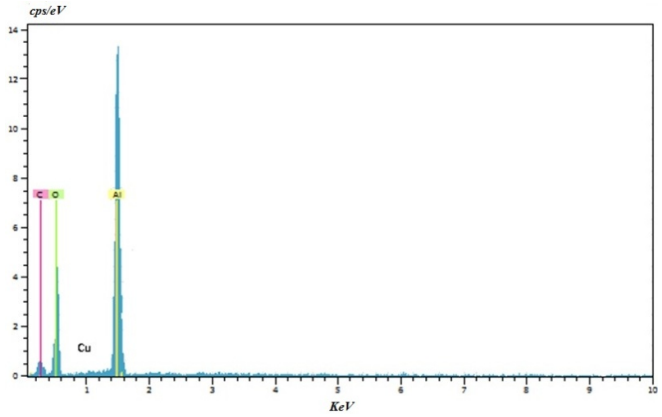

Figure 6 . Energy dispersive X-ray of MPA 5%CNT

3.3. Cell Culture Test and Cell Cytotoxicity

Mesoporous alumina and carbon nanotubes are extensively applied in biomaterials field; but, to date, no reports has been done on the use of composite of these two materials in medical applications. So, to ensure non-toxic effect of composite and cell viability, cell culture test (MTT assay) is performed on samples with 3 and 5 weight percent of carbon nanotubes. The MTT assay is a colorimetric assay for measuring activity of cellular enzymes that reduces and breakdowns the tetrazolium yellowish crystals by the enzyme succinate dehydrogenase and forms an insoluble blue crystals.

In this method, in contrast to other methods, cell picking and washing steps, which often lead to loss of some cells, are eliminated and all the test steps are done in the same micro-plate; thus, this method has high repeatability, accuracy and sensitivity.The results of cell culture of the samples with 3 and 5 % of CNTs are given in the Fig.7

According to these tests, if percentage of viable cells in the culture medium is above 80, this composite can be considered as non-toxic materials and it can be used for other biological tests.

Another advantage of using these nanocomposites in biological applications is their easy sterilization process because these nanocomposites can be exposed to sterilizing temperatures without any problems.

3.4. Fourier Transform Infrared (FTIR) Spectroscopy

For composite nanoparticles disperse in a neutral aqueous solution, free O and Al atoms on the surface nanoparticles attract H^+ and OH^- ions, respectively, and produce OH-rich surfaces. Hydroxyl groups can interact with APTES

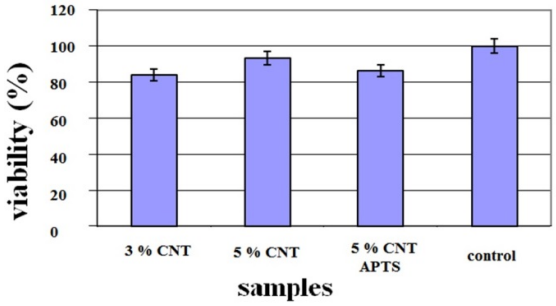

Figure 7. Cell culture results

molecules, as schematically depicted in Fig. 8 .

So, nanoparticles can be coated with APTES molecules via chemical bonds. CNTs also have carbon atoms on their surfaces that can attract oxygen, hydrogen, nitrogen atoms or other functional groups. So, infrared spectrum of the nanotubes can be also observed. To compare and evaluate use of APTES solution, infrared spectroscopy experiments are done in the samples with 0, 3 and 5 % of carbon in both functionalized and non-functionalized samples (Fig.9).

FTIR diagram of the porous alumina with APTES shows distinct peaks at 1065, 1470, 1562, 2814, 2930, 3350 and 3400 cm^{-1} that are in good agreement with the peaks reported for APTES. CH_2 groups are at wave numbers 2814 and 2930 cm^{-1} which are attributed to asymmetrical and symmetrical stretching vibrations of C-H bonds. The peak at 1470 $cm^{-1}$is 1562 and is due to vibrations of $N-H_2$ amino groups. Weak peak in the vicinity 1065 cm^{-1}indicated asymmetrical stretching vibration of Si-O-Si bonds.Broad peak around 1630 and 3400 cm^{-1} related to moisture absorbed on surfaces of particles, OH hydroxyl group was observed in both cases as well. Existence of these peaks proves APTES on surface of the nanoparticles [4].

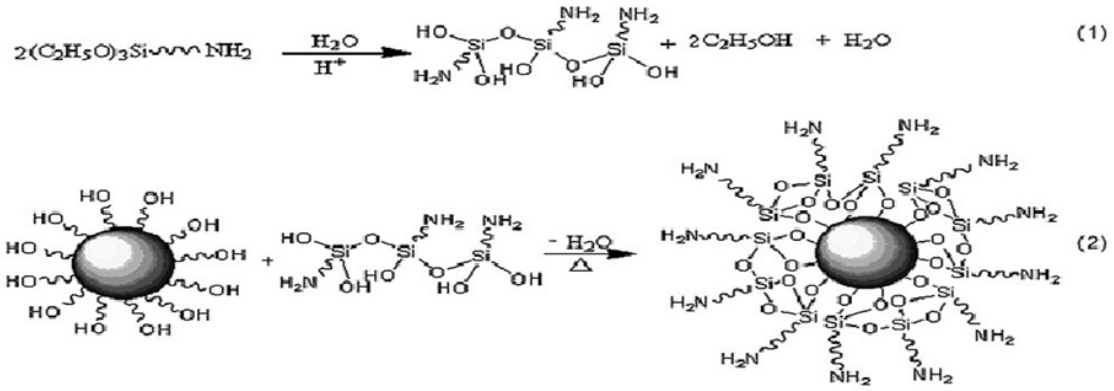

Figure 8. Interaction of OH groups with APTES

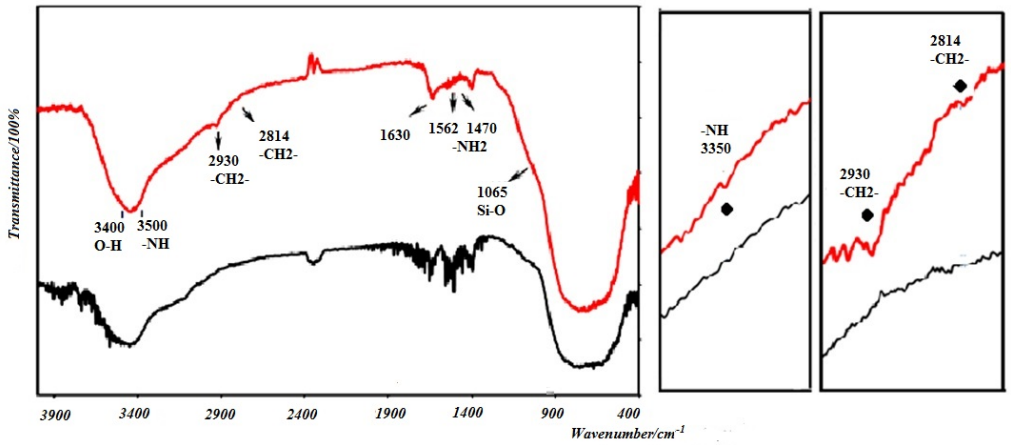

Figure 9. FTIR diagram of the porous alumina with and without APTES

3.5. Drug Loading

Introducing carbon nanotubes to a porous matrix partially causes disordering of mesoporous structure. As mentioned above, this disordering results in decreased particle size as well as high surface area of CNTs, both of which cause increase in specific surface area of the nanocomposite.During drug loading, these properties play an important role because attaching drug molecules to the surface depends on the number and type of surface molecules; therefore, as expected, with increasing CNT amount in the composite, drug loading also increases. The results are shown in Figure 10.

When nanocomposites are functionalized by APTES solution, the amino functional groups are formed on the inner and outer surfaces of the pores; this phenomenon somewhat reduces inner diameters of pores, which might reduce the available space and thus might reduce the amount of loaded drug. But, as can be seen from results of the experiments, the functionalized samples are aabsorbed into significantly more drugs [4].

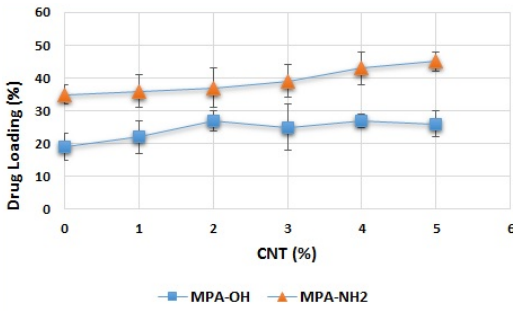

Figure 10. Drug loading in nanocomposites

3.6. Drug Release

IBU release profile from MPA/CNT in PBS (pH=7.4) at 37 °C is shown in Figure 11.The release rate is high at early intervals, because in those intervals the diffusion pathways are short, resulting in high concentration gradients (driving forces for drug diffusion) and, thus, high drug

release rates. With increasing time, the length of the diffusion pathways increases, resulting in decreased drug concentration gradients and, thus, decreased drug release rates [14].

The initial fast release may be due to release from exogenously bounded drugs on the surface of the tablets. During the loading and release process, the IBU molecules can be adsorbed onto the surface of porous materials in the impregnation process and released by diffusion-controlled mechanism [15]. Burst release may be explained in two different aspects: positive and negative. Positive aspect focuses on situations in which rapid release provides high drug concentration that is desirable at the beginning of wound treatment

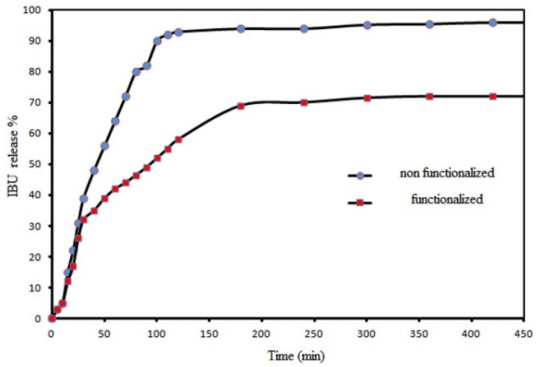

Figure 11. Kinetic profile of IBU release from Al_2O_3/CNT into PBS (pH=7.4) at 37 °C

In these cases, an initial burst provides immediate relief followed by aprolonged release to improve subsequent healing. The negative aspects of burst release are economic and therapeutic waste of drug [16].

Interaction strength between surface molecules and ibuprofen depends on type of chemical elements on the surface of particles. Ibuprofen release kinetics from the composite also follow a similar trend so that, the stronger the interaction between particle surfaces and ibuprofen, the slower the release rate would become. In the case that only chemical elements presented in the surface have been hydroxyl groups and the samples did not been functionalized by APTES yet, they have demonstrated higher release rate than functionalized state. The interactions between OH groups on the alumina surface and COOH groups on ibuprofen are weaker than those between NH_2 groups of ATMPS and COOH. Significant release of ibuprofen happenes when an adequate amount of solvent penetrates into mesoporous channels to solve the drug; so, as these channels became much bigger, release rate of ibuprofen is higher.

When the composite is functionalized by APTES, it partially covers surface of the pores and, besides chemical effects, reduced pore size; so, another reason for slower release rate is reduction in inner diameter of functionalized pores and steric hindrance created by APMTS compared with OH's [4, 5].

4. Conclusions

A new facile and reproducible method was developed for the onepot synthesis of well ordered mesoporous alumina/carbon nanotube composites. According to the current study, it seems that, by adding carbon nanotubes to the composites, hexagonal structure of alumina mesoporous is somewhat disturbed and specific surface area of the nanocomposites increases with reduced particle size. X-ray diffraction diagrams of nanocomposites confirmed the mesoporous structure. Scanning Electron Microscope (FE-SEM) images showed relatively uniform distribution of spherical nanocomposite powder with particle size of less than 100 nm. The results of cell culture test revealed non-toxicity and usability of this composite as a biomaterial and paved the way for more biologic investigations.

Drug loading measurements showed that, as the amount of CNTs increased in the composite, ibuprofen loading increased, which could be due to increasing surface contact and interactions between drug molecules' nanocomposites. Through surface functionalization of nanoparticles by amine functional groups, drug releasing showed slower rate than the initial state, which indicated stronger interaction between the amine groups and ibuprofen molecule compared with hydroxyl groups.

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