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Synthesis of PVA/γ-Fe_2O_3 Used in Cancer Treatment by Hyperthermia

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***Abstract*—**In recent years a new method of combination treatment for cancer has been developed and studied that has led to significant advancements in the field of cancer therapy. Hyperthermia is a traditional therapy that, along with a creation of a medically approved level of heat with the help of an alternating magnetic AC current, results in the destruction of cancer cells by heat. This paper gives details regarding the production of the spherical nanocomposite PVA/γ-Fe_2O_3 in order to be used for medical purposes such as tumor treatment by hyperthermia. To reach a suitable and evenly distributed temperature, the nanocomposite with core-shell morphology and spherical form within a 100 to 200 nanometer size was created using phase separation emulsion, in which the magnetic nano-particles γ-Fe_2O_3 with an average particle size of 20 nano-meters and with different percentages of 0.2, 0.4, 0.5 and 0.6 were covered by polyvinyl alcohol. The main concern in hyperthermia and heat treatment is achieving desirable specific absorption rate (SAR) and one of the most critical factors in SAR is particle size. In this project all attempts has been done to reach minimal size and consequently maximum SAR. The morphological analysis of the spherical structure of the nanocomposite PVA/γ-Fe_2O_3 was achieved by SEM analyses and the study of the chemical bonds created was made possible by FTIR analysis. To investigate the manner of magnetic nanocomposite particle size distribution a DLS experiment was conducted. Moreover, to determine the magnetic behavior of the γ-Fe_2O_3 particle and the nanocomposite PVA/γ-Fe_2O_3 in different concentrations a VSM test was conducted. To sum up, creating magnetic nanocomposites with a spherical morphology that would be employed for drug loading opens doors to new approaches in developing nanocomposites that provide efficient heat and a controlled release of drug simultaneously inside the magnetic field, which are among their positive characteristics that could significantly improve the recovery process in patients.

***Keywords*—**Nanocomposite, hyperthermia, cancer therapy, drug release.

I. Introduction

SMART materials are considered as a new generation of materials that are better than the previous structural and functional cases. These material scan accept external stimulation such as loading or environmental changes such as temperature, humidity, various pH levels, or magnetic or electric fields due to their innate intelligence [1]. Magnetic materials are one of these smart groups of materials that have a special place in the diagnosis and treatment of cancer. Medical uses of magnetic powders dates back to the time of ancient Greece and Rome, but fundamentally and scientifically have been used since 1970 in biology and medicine. Magnetic nanoparticles are used in abundance for targeted delivery of medical agents and act based on magnetic drug targeting (MDT) which includes a strong affinity between ligand and receptor or through magnetic attraction of a particular tissue [2]. MNPs are considered significant for having possibility to remote control of therapeutic agents in transferring particles to target texture and thereby are called magnetic targeted carriers (MTC). Magnetic particles are available in two main forms in medical applications:

1. Core-shell structure: As a metal core coated with biocompatible materials and are most of interest due to more easiness to prepare them for applications and better controllability.

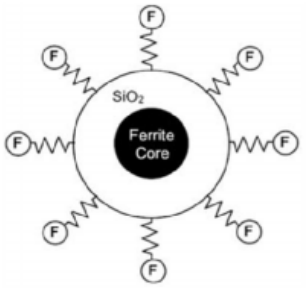

Fig 1 Core-shell sample coated with silica or polymers such as PVA or dextran

In a kind of MNP having core-shell structure the iron core of magnetic oxide in form of Magnetite (Fe_3O_4) or Maghemite (γ Fe_2O_3) and the shell structure is composed of materials such as silica, Dextran, PVA or metals like gold, and these materials provide functional groups attachment using cross-linker. These particles will be synthesized using ionic and nonionic surfactants or by encapsulating the inside the structures such as carbon cages or ferritin proteins and finally will possess functional group by binding carboxyl, amine, biotin, streptavidin and antibody groups [3].

2. Located particles in a porous polymer: They are in the form of biocompatible porous polymers that magnetic nanoparticles have been inserted inside them. The advantage of this method is production of particles with a relatively narrow size distribution and particular spherical morphology [4].

Among the common problems in making nanocomposites according to previous researches, non-uniform distribution of nanoparticles in the background material or non-uniformity of

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the particles size can be pointed out that each of which created some problems in generating heat for the treatment of hyperthermia [6].The origin of these problems is due to a low specific absorption rate. This rate is in fact as a factor that defines the electromagnetic energy absorbed by a mass unit of biological material. Best mode for the highest efficiency in heat therapy is obtaining the maximum specific absorption rate [7]. SAR is depended on various parameters such as magnetization, particles size and particles size distribution, magnetic field strength and electric field frequency. The unit of this rate is defined as $\frac{Watt}{Kg}$and is proportional to the rate of temperature increase. For adiabatic state equation is as follows [8]:

$$SAR = 4.1868\ {}^{P}/_{M_c} = C_e\ {}^{dT}/_{dt}$$

where P is electromagnetic power absorbed by the sample, M_c is the sample mass and C_e is the specific heat capacity of the sample [8]. Particles size affects the stable time of each mechanism. Particles larger size will lead to larger *Brownian* and Neil's relaxation time function. For increased security of magnetic nanoparticles, their intake by normal cells should be significantly lessened. Due to temperature of 42C° to 45°C in Hyperthermia, normal cells will not suffer damage.

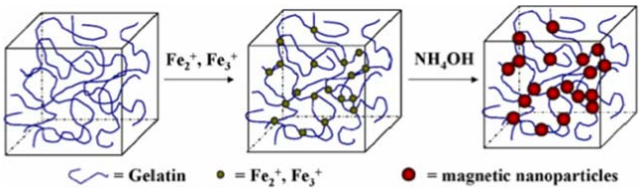

Fig 2 The presence of metal ions as a factor for creating magnetic property in polymer composite [5]

Treatment of cancer using heat generation in the tumor region to destroy cancer cells is considered as the main applications of smart magnetic nanocomposites. The root meaning of the word hyperthermia stems from heat and the second part 'thermia' stems from treatment. And the use of this word actually implies treatment based o heat generation at the tumor site [9]. This method involves increasing temperature, in the site of the tumor that will lead to the physiology change of patient cells and ultimately cell death (apoptosis). This method complements traditional and modern methods, such as chemotherapy, radiation therapy, surgery, gene therapy and immunotherapy for cancer [10].

II. Materials and Methods

Iron oxide nanoparticles were purchased from Tecnan Co, Spain. Polyvinyl alcohol (*Muhyul* 28-99- 145000 gr/mol) for synthesis was purchased from Sigma–Aldrich Co, St Louis, MO, USA. Ether-petrol, sodium sulfide and borax sodium as a chemical crosslinker was purchased from Sigma–Aldrich Co. Ethanol acid and sulfuric for analysis were purchased from Merck Schuchardt OHG, Germany and eventually Span 80 as a emulsifier was obtained from Merck Schuchardt OHG, Germany.

A. Synthesis of Magnetic Nanocomposites PVA / γ-Fe_2O_3

Nanocomposite was prepared using emulsion phase separation method. Initially, 1.6 grams of powdered polyvinyl alcohol (PVA) was dissolved in 20ml double – distilled water to prepare a solution of 8% PVA. And solution was placed on a magnetic stirrer at a temperature of 80°C and at a speed of 1200 rpm for 15 minutes .Concurrent with the preparation of polymer solution, liquid paraffin in volume of 20mlwas placed on another stirrer in the same situation of polymer solution. For making corresponding nanocomposite a crosslinker factor was required that the Borax solution was used for this purpose and a Borax solution of 15% was prepared (20 ml doubled-distilled water was placed on a magnetic stirrer at a speed of 1000 rpm and at temperature of about 80C° and 3 grams of Borax white powder was added to it slowly and after 15 min the solution was quite ready). After preparing raw materials, the paraffin oil solution was placed under homogenizer at a speed of 20000 rpm and after 1-2 minutes the iron oxide nanoparticles gained weight as 0.2 of weight percentage of the oil. After the uniform dispersion of nanoparticles, PVA polymer solution of 8%, was added slowly and with a pipette into the oily phase as drop wise and as much as 2 ml. In this step as much as 0.01% of Span 80 was added as emulsifier. Prepared sample was placed at different speeds of homogenizer for 20-25 minutes at times 5 minutes, 10 minutes, and 15 minutes at speeds 20000, 15000 and 10000 respectively.

Then, the sample was placed into a pool of cold water at a temperature of 5C° for 5 minutes and then laid back down homogenizer and at speed of 5000 rpm to 8000 rpm Borax solution was drip slowly using the pipette into the sample that was being provided. After 30 minutes, the sample was removed from under the homogenizer and after 1-2 min sample was placed again on the magnetic stirrer with speed of 600 rpm and the temperature of 50C° for 3 hours and was exposed to following conditions:

Centrifuged for 8 min, 8000 rpm and temperature of 24C° and then removed from the centrifuge, the oil phase was separated thoroughly and the obtained powder at the bottom of the container, was rinsed for being dehydrated with extremely diluted acetone and then for fully washing the oily phase, the resulting powder was washed 3 times in 100ml of ether-petrol solution and was held for 4 hours after passing through the filter in the oven with temperature of 50C° and ultimately powders of about 100 to 200 nm were obtained.

III. Characterization

Powdered polymer samples containing nanoparticles were analyzed to investigate its surface morphology and determine the average particle size of powder by Scanning Electron Microscope SEM (KYKY EM3200), and for the purpose of nanocomposite particles size distribution measuring, Dynamic light scattering (DLS) test (Zetasizer model ZEN 1600) was done. To evaluate the composite compounds, FTIR test was conducted on samples to examine the structure and chemical

bonds and the resulting peaks. Structural changes of the samples were examined using the model analyzer NEXUS Thermo-Nicolet 870 in the range of 400-4000 cm^{-1}. Besides, in this test the magnetic properties of γ-Fe2O3 and its nanocomposite at different percentages of 0.2, 0.4, 0.5, 0.6, 0.8 and 1 ,with polyvinyl alcohol using Vibrating sample magnetometer (VSM) (Magnetic Daghigh Kavir Co. Iran) were obtained and analyzed in which the maximum magnetic field was equal 8000 Oe and AC magnetic susceptibility measurements in the magnetic field was about 10 Oe and was performed at different frequencies between 30 to 1000 Hz.

IV. Results and Discussion

A. SEM Test

According to the SEM images taken from the nanocomposite samples with different concentrations of iron nano-oxides, it is concluded that using the developed method that is considered a kind of emulsion phase separation method we can reach very suitable shell-core structures with particles size of about 120 nm and spherical morphology that is regarded as one of the main goals of this project.

Fig 3 SEM images of PVA/γ-Fe_2O_3nanocomposite samples at variety concentrations with the average size of 200 nanometers

The homogeneity of the particles shape and size according to researchers' previous reports and studies, have very important role in enhancing the specific absorption rate and treatment efficiency of Hyperthermia and drug magnetic release and hydrophilic property in this project these objectives were achieved. With regard to particles size and high surface to volume ratio they are expected better heat efficiencies. On the other hand through the PVA structure in the shell of this nanocomposite and hydrophilic property and the absence of surface charge and biocompatibility of this substance much smaller toxicity is anticipated compared to iron oxide nanoparticles with no coating.

B. FTIR

First, in Fig. 4, and at the polyvinyl alcohol items, a prominent peak is observed in the distance between 3400 cm^{-1} to 3500 cm^{-1} that is corresponded to alcoholic OH bond. The peak related to wavelength of 2900 cm^{-1} is corresponded to stretching CH bond and the wave number peak of 1650cm^{-1} is corresponded to C-C bond. About items corresponded to γ-Fe2O3 peaks available at wavelength 3400cm^{-1} and 1640 cm^{-1}, are related to tensile and flexural O-H bonds that are formed due to water absorption on the surface of the nanoparticle and index peaks present at wavelengths of 560 $cm^{-1}$and 680 cm^{-1} are related to Fe-O bonds (in spinal vibration modes). In items related to PVA-γFe2O3 nanocomposite in Fig. 4, index peaks are consisting of: the peak related to O-H at a wave number of 3350 cm^{-1} that compared to the alcoholic OH bond of polymer, has been shifted a little to the rightward, and especially downward that is reflecting increased OH concentration and that is due to the use of cross-linker Borax (chemical formula= B(OH)4) and thus OH groups increase in composites or is due to moisture absorption. But the remarkable point is the peak related to B-O-B, Borax bond is observed at the wave number of 1400 cm^{-1} and possesses small concentration and also the peak of the wave number of 952cm^{-1} which is related to B-O indicates the binding of O-H borax groups to PVA chain or another borax (and also) is representing the chemical bonding of borax with polymer, and also due to the peak (Fe-O-C) that is completely particular in wave number of 1092 cm^{-1} we can absolutely certain that a hydrogen bond between the hydroxyl groups of polyvinyl alcohol and iron oxide surface exists.

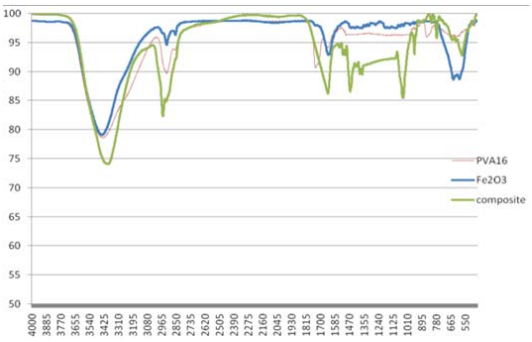

Fig. 4 FTIR spectrum for PVA, γFe_2O_3 and PVA-γFe_2O_3

C. VSM Test

Based on VSM test results on composite PVA-Fe_2O_3Saturation magnetization of non-coated case was

40emu/g. As it is represented in the chart for nanoparticles coated with polyvinyl alcohol, saturation magnetization increases with increasing concentration of Fe_2O_3. This event decreases with the increase of polyvinyl alcohol percentage, which has different reasons. It may be due to the effects of diluted adsorbed water or hydroxyl groups of polyvinyl alcohol or it may be due to very small volume fraction of anti-ferromagnetic amorphous iron oxide. The reduced magnetism may also be due to particle small surface is, and this is because of irregularities in the nuclear spins of the surface. Surface effects are particularly important for small particles. Because the smaller particles become, the more the number of atoms on the surface will be. By observation to similar studies, it was derived that because, γ-Fe_2O_3 is a ferro-magnet material we can see more heat losses than Fe_3O_4. Because due to being a super magnet material they cause less hysteresis at the time of removing magnetic field compared to γ-Fe_2O_3.Therefore, γ-Fe_2O_3 indicates higher efficiency in Hyperthermia conditions compared to super paramagnetic nano-oxide of iron oxide.

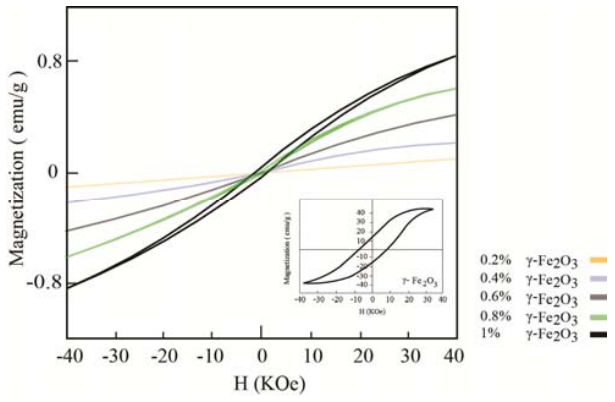

Fig. 5 Magnetic behavior of the γ-Fe_2O_3 particle and the nanocomposite PVA/γ-Fe2O3 in different concentrations

D.DSL Test

The DLS test typically is used for measuring diameters will be in the 100–300 nm range with a polydispersity index of 0.3 or below. The presence of the larger particles will dominate the light scattering signal and mask the presence of the smaller particles. When submitting a colloidal dispersion for DLS analysis, it is important to submit enough material in order to obtain a sufficient signal and have high quality data. The volume of sample submission for DLS analysis was 1-2mL and moreover sufficiently concentrated. Alternatively, 100-200uL of highly concentrated sample was submitted and diluted to 1mL. Sample was run multiple times and made 6 separate DLS measurements with each measurement consisting of 5 sub-runs with 10 seconds duration. As it is shown in Fig. 6, average size of nanocomposite is between 120-240 nm. Furthermore the size distribution of particles shows nearly homogeneity.

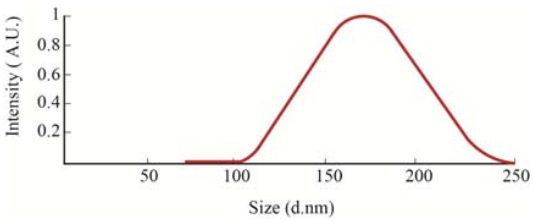

Fig. 6 Dynamic light scattering analysis for measuring nanocomposite particles size distribution

V. Conclusions

In the project carried out, spherical nanocomposite PVA/γ-$Fe_2O_3$with dimensions less than 120 nm was obtained in order to achieve a high thermal efficiency and a uniform distribution of temperature in cancer therapy using Hyperthermia method and in this process emulsion phase separation method was used. In order to evaluate the morphology and particles size SEM and DLS test were used. Also to check the connections and the chemical bonds nanocomposite, FTIR analysis was performed and the corresponding peaks are demonstrating the borax OH groups to connection to PVA chain and (Fe-O-C) peak that was located at wave number of 1092cm^{-1} that was a characteristic of a hydrogen bond between the hydroxyl groups of polyvinyl alcohol and iron oxide surface. Finally to determine the magnetic behavior of γ-Fe_2O_3particle VSM test was conducted and the obtained curve showed a remarkable thermal hysteresis that confirmed the suitability of this particle for use in hyperthermia. Hyperthermia can be said in a general conclusion based on magnetic nanoparticles can both be used to generate an intense localized heat and in line with it to be applied for use in a polymer platform with holes for releasing encapsulated drugs. Although research in this area is very extensive, but still there are many challenges and deficiencies that need to be investigated, and several experiments. In this project, it has been tried a lot to challenge this issue from other aspects that have not still been investigated.

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