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Research Article

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Synthesis and Formulation of Powerful ZnO Q-Dots/CNTs@Fe_3O_4 Nanopackage as a Predominant Anti-HIV-1 Infection

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Abstract

Usage of fine and uniform Fe_3O_4 nanoparticles (NPs) including super-paramagnetic unique properties developed state of the nanobio-formulations in recent years. We have shown a new formulated nanocomposition of super-paramagnetic iron oxide (Fe_3O_4) NPs (as substrate) with carbon nanotubes (CNTs) (as activator). Besides, ZnO@CNTs was synthesized as a magic assistant/hybrid for ZnO quantum dots nanoparticles (Q-Dots NPs) in this nanopackage. This novel formulated water-based nanofluid product consists of strong stabilizer, suitable dispersant-wetting agent complex and desirable water in oil emulsifier (w/o) package to damage HIV infection (AIDS) type 1. The achieved results demonstrated that smart nanofluid formulation had excellent functions as inhibitor, controller and treatment (Antiretroviral therapy (ART)) for HIV-1 integrase and could act as strong oxidizing agent. The nanofluid product was completely characterized with SEM morphology, TEM images, FTIR spectroscopy, XRD pattern, UV–Vis absorption spectroscopy, EDS and mapping of internal layers for one of the SEM surface morphology. Moreover, HIV-1 replication Assay, RT (reverse transcriptase) Assay, integrase assay, and cytotoxicity tests were performed and compared with Zidovudine (ZDV) and Raltegravir (RAL) as control antiretroviral medications. The specific interaction of this nanopackage with the target RNA and DNA proteins has been very interesting through main redox reactions.

Keywords: AIDS; HIV-1 infection; Nanohybrids; ZnO Q-Dots nanoparticles; Anti-retroviral agents; Thiol/disulfide redox state; Nano-oxidizer; Nano- reducing agent; DNA and RNA proteins.

Introduction

Human immunodeficiency virus (HIV) exterminates the fighting CD4-positive T lymphocytes (the host cell receptors) as a subgroup of white blood cells that naturally regulate the immune responses to infection [1]. HIV virus spreads throughout the body using T cells to replicate itself, and at the same time it reduces the number of cells that the body needs to defend itself. HIV was firstly discovered by Frenchman Luc Antoine Montagnier and American Robert Galloway [2]. Acquired immunodeficiency syndrome (AIDS) which is known as the most advanced level of HIV infection, became an increasing global health, economic and social concern due to the continued evolution of drug resistance strains [3]. As a warning figure, a static on the word epidemic of HIV/AIDS by United Nations Program on HIV/AIDS (UNAIDS), reported that 37.9 million people were hardly living with HIV/AIDS in 2018 [4]. In response, nucleoside-analog reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), fusion inhibitors, integrase inhibitors, protease inhibitors (PIs), and co-receptor antagonists are mainly and available drugs for therapy of HIV-1 infections [5-8]. Plasma membranes of CD4+ T cells could also be collected and coated onto nanoparticle cores and act as decoys for viral attack and neutralize HIV virus.

Nanotechnology has emerged as a powerful approach for treatment and prevention of HIV/AIDS due to many important attributes including ability to increase circulation or tissue retention time, encapsulate various materials, improve solubility and bioavailability, sustain drug release, enhance drug potency and decrease or side effects or toxicity [9-11]. Specially, several nanotechnology-based delivery systems have been developed in order to improve HIV therapy, specifically nano emulsions,

polymeric micelles. inorganic NPs such as silver, gold, iron, and zinc oxide, liposomes, dendrimers, solid lipid NPs, graphene oxide, nanocrystals, nanocapsules and drug conjugates (e.g. with low-density lipoproteins or peptides) [12-17], whereas the emphasize is always on smart technology of nanocarriers or obedience of nano formulations to target particular tissues or cells. Herein, the magnetic drug delivery mechanism has received highlighted attention, using a magnetic field to direct the drug to the exact biological target, while other healthy tissues of the body are free from drug damage. Meanwhile, superconductive iron oxide nanoparticles (Fe_3O_4 NPs) due to biocompatibility, biodegradability, suitable dispersion in water-based environments and super-magnetic properties would make drug carriers better than other systems mentioned [18-21]. The new goal of the researchers is to synthesize special quantum dots NPs capable of attaching to viruses and preventing them from entering into cells. Because, these NPs are not toxic to cells and can bind up to HIV GP120 protein (or gp120). Then, it is significant points related to the NPs which can selectively bind up to GP120 (a key envelope glycoprotein of HIV-1 or a part of the outer layer of the virus) and prevent GP120-induced killing of bystander CD4-positive T lymphocyte (CD4+ T) receptor on a host cell and neutralize HIV infectivity [1,5,18,22]. Herein, scientists believe GP120 binding to α-Galcer (alpha-Galactosylceramide protein found on the membrane of many cells) is important for sexually transmitted HIV infection [5].

Identifying a powerful hybrid system for surface modified-carbon nanotubes (CNTs)@Fe_3O_4 nanocomposites, in a systematic order the ZnO/ modified-CNTs and ZnO Quantum Dots (Q-Dots) NPs in a very stable water-based nanopackage used for inhibition of human immunodeficiency virus type-1. Hydrophilic surface of multi-walled carbon nanotubes (MWNTs) can dope into the iron oxide Fe_3O_4 super-paramagnetic nanoparticles and makes it magic nanocomposites (due to their high biocompatibility in the body). On the other hand, ZnO @CNTs/fatty acid nanohybrids provide reactivity and responsiveness to media, holding ZnO Q-Dots NPs in stable and uniform water-based valuable nanofluid product for this work. Our synthesized antiretroviral nanodrug smartly used to prevent, control and successful treatment of HIV/AIDS infection, whereas biological activates such as zidovudine (ZDV) and Raltegravir (RAL) as nucleoside analog reverse-transcriptase inhibitor (NRTI) used as references. Introducing a promising platform for nanomedicine applications, our designed ZnO Q-Dots/CNTs@Fe_3O_4 nanopackage which equipped with the power of redox reactions, can decrease the amount of HIV in body and highly improve the immune system, since the proteins of cell surfaces could adsorb onto this package of nanoparticles and nanohybrids, inducing oxidation of cysteines and methionine residues of the proteins. The success of smart NPs will occur when the NPs have both suitable structure and size to attach to the GP120 protein (in HIV) and do not let the HIV-1virus to bind to the CD4 receptor of the host cells. Indeed, the "prevention" property of NPs are in priority compared to "treatment" and "control". The quantitative measurements of the relative safety of nanodrug have presented a magic anti-HIV agent for the first time.

Material and Methods

2.1. Synthesis of original nanofluid product including four steps

2.1.1. Synthesis of Hydrophilic surface of multi-walled carbon nanotubes (MWNTs)/ Fe_3O_4 as a potent nano-oxidizer

Multi-walled CNT (6-8nm) which its surface was functionalized with –OH groups was purchased from Neutrino Company in Iran. An appropriate amount of CNT NPs was mixed with super-paramagnetic iron oxide NPs from mixture of (Fe_2Cl +Fe_3Cl) by coprecipitation method [23,24]. In order to make a powerful nanohybrid, the candidate distilled water, absolute alcohol, ionic surfactant and wetting agent (oleic acid ethoxylated from Kimyagaran Emrooz Company Tehran, Iran) thoroughly mixed under vigorous magnetic stirring at 60–70 ºC. The reaction mixture was then refluxed at 70–80 ºC for 4-8 h to complete the reaction. Then, hydrothermal process was maintained at 80 -90 ºC for 48 h. This nanoproduct was completely evaporated and also purified and finally was dried at 100-150 ºC.

2.1.2. Synthesis of ZnO@CNTs/Fatty acid as an efficient nano-redox agent

This nanocomposite was made by co-precipitation containing 4-7 gr. zinc acetate. $2H_2O$ (Sigma Chemical Co., St. Louis, MO) and 2-5 gr. multiwalled CNT (8-15 nm) which were dissolved in water-alcohol solution mixture. The hydrolysis reaction was carried out by adding KOH and urea solutions, and the pH (10-11) of the solution was adjusted at temperature of reflux for 4-5 h under vigorous stirring. The addition of polar stabilizer non-ionic surfactant is very necessary during reflux reaction. The obtained nanoproduct was placed in an oven (80-90ºC) for 48h under hydrothermal process, where the solvent was then completely evaporated at 70-80ºC and purified at cold temperature. The crucial surface of nanocomposites was modified with green and eco-friendly fatty acid or fatty oleic acid (Merck Co.) through wet chemical reaction method in water-based solution [25,26].

2.1.3. Synthesis of ZnO Q-Dots nanoparticles as a strong nano-oxidizer

In this synthesis, we use zinc nitrate and zinc chlorate mixture which dissolved in cold bath and added to a cold mixture of some important reagents like wetting agent, glycerin solvent, ethoxylated emulsifier, acetylacetone (acac) from Sigma-Aldrich as a chelating agent solvent for making assistant chemical compound in sol gel, using chemical alkaline hydrolysis method. It is demonstrated that acac avoids the fast hydrolysis of zinc salts, that provide certain benefits to form very stable sols in cold temperature and fast quenching process at long time. After the mixing, they were heated to reflux quickly at 70°C in the hot bath for 6-8 h under vigorous

stirring [27]. The obtained product was immediately evaporated, washed and it was well purified. Finally, the precipitate was dried at 80-100°C in oven and calcined at 700°C for 6h.

2.1.4. Fabrication of New nanofluid Product as nonohybrids product

Of around 4-6 gr. CNTs/Fe_3O_4 nanocomposite was dissolved in 20 ml absolute alcohol and the solution was mixing with ZnO/CNT nanoproduct in suitable solvent at room temperature. ZnO Q-Dots NPs was added to PEG (polyethylene glycol 4000-6000) solution as a binding and stabilizer agent and also a magic solvent for achieving new smart energetic surfaces of these various NPs. Also, due to having a much more favorable suspension and physical and chemical stability of homogeneous nanofluid product at the optimized pH value of 7–8, here, it was necessary to add the additive of polysorbate 80 (Merck Co.) as an effective nonionic surfactant and W/O emulsifier for fabrication of the water-based nanoformulation original product. The AIDS virus is highly sensitive to environmental acid and base changes. pH below 7 and above 8 causes the virus to become unstable. For this reason, the pH nanofluid of the manufactured product was adjusted to 7-8. Figure 1 can demonstrate the systematic total process during of fabrication of this nanopackage fluid product as a surprising nanomedicine strategy in antiretroviral nanodrug.

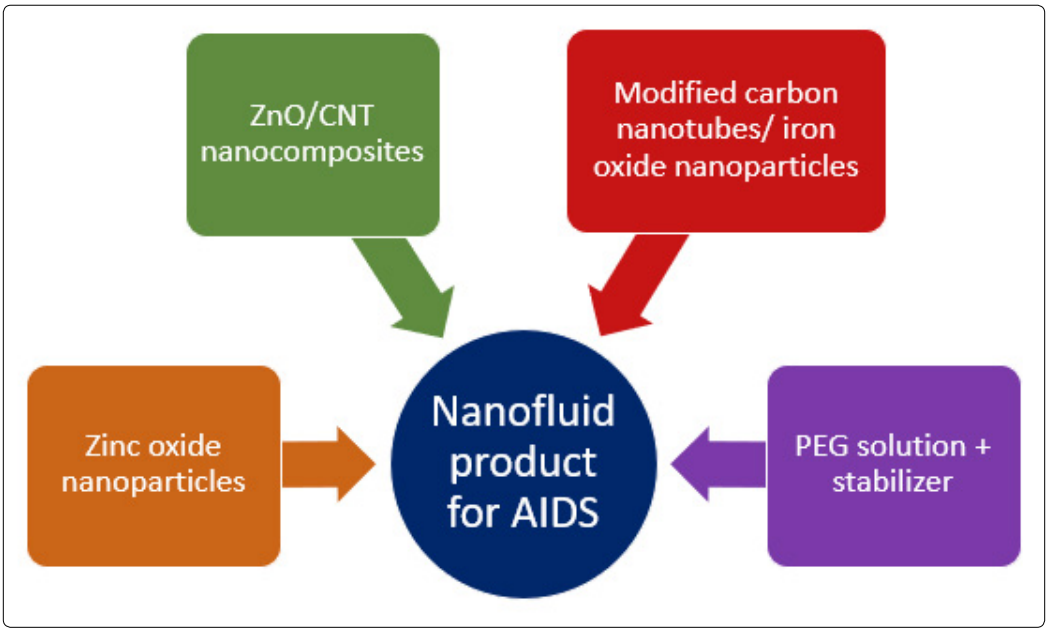

Figure 1: The Final Protocol in Selection of Suitable Basic Nanomaterials in Synthesizing Nanofluid Product Against HIV/AIDS.

2.2. HIV-1 replication inhibition assay

Human embryonic kidney (HEK) and Hela cells were obtained from the National Cell Bank of Iran. HEK293T cells were cultured and maintained in Dulbecco's modified Eagle's culture medium (DMEM, Sigma, USA) supplemented with FBS (15 %), penicillin (100 U/ml), and streptomycin (100 lg/ ml). To generate HIV-1 single cycle replicable (SCR) pseudotyped form with vesicular stomatitis virus glycoprotein (VSV G), pMD2G, pmzNL4-3, and pSPAX2 plasmids were used in this study. Briefly, these plasmids were simultaneously transfected into HEK293Tin 6-well plates (4×10^5 cells/well) in certain proportion using Lipofectamin 3000 reagent (Thermo Fisher Scientific, USA) according to the provided protocol. At 48 and 72 h after transfection supernatants of the transfected cells were harvested, and after filtration with 0.22 µm filters, mediums containing viruses were mixed together and concentrated for 4 hours in 60,000g in 4 °C by ultracentrifugation. The virions pellet was shaken gently overnight in 1/30 volume of RPMI 1640 at 4 °C and the quantity of the HIV-1 was measured using HIV Type1 p24 Antigen ELISA (ZeptoMetrix Corporation, USA) according to manufacturer's instruction as a major indicator of oxidative stress.

To evaluate the anti-HIV-1 activity of the above-mentioned NPs, Hela cells at the density of (8×10^3 cells/well) seeded in a 96-well plate containing 200 µl of culture medium. The day after, SCR HIV virions with the concentration of 600 ng p24 were added to each well. After six hours' post-infection, the cells were washed twice with fresh medium and the nanoparticle compound were added in five concentrations (5, 10, 50 and 100 µM) to the wells as triplicate. Three days after inoculation, the supernatant was collected and the HIV-1 P24 level of the supernatant of each well was determined by quantitative HIV-1 p24 Antigen ELISA. p24 ELISA is a standard sandwich enzyme-linked immune-sorbent assay. The 50% effective dose concentration (EC50) was defined according to the percentage of infectivity inhibition relative to the positive control (Zidovudine (ZDV) and Raltegravir (RAL)).

2.3. Cytotoxicity of nanofluid Product against Hela cells

The cytotoxicity of the nanofluid product on Hela cells was measured using the XTT proliferation assay kit II (Sigma-Aldrich). The XTT assay is used to measure cellular metabolic activity as an indicator of cell viability, proliferation and cytotoxicity such as human cervical cancer cell lines (HeLa). Only in living cells mitochondria are able to reduce XTT to form an orange colored water-soluble dye. Therefore, the concentration of the dye is proportional to the number of metabolically active cells, which in that reaction the XTT tetrazolium changes to XTT formazan. For this purpose, the effects of nanofluid product on the viability and proliferation of Hela cell lines treated with various concentrations (1000, 500, 100, 10 µM/ml) of compounds for 72 hours. The degree of selectivity of the compounds can be expressed by its Selectivity Index (SI) value. The selectivity index (SI) is a quantitative measurement of the relative safety of a drug which was calculated using equation SI = CC50/EC50. SI=LC50 of pure compound in a normal cell line/LC50 of the same pure compound in cancer cell line, where LC50 is the concentration required to kill 50% of the cell population. Usually, high SI value (>2) of a compound gives a selective toxicity towards cancer cells.

2.4. HIV-1 integrase Assay

To measure the inhibitory activity of our compounds on 3 beta-processing and strand transfer reaction of HIV-1 integrase (IN), we used a commercially reliable integrase kit (HIV-1 Integrase Assay Kit version 3.0, XpressBio Life Science Product, US). Following manufacturer's protocol**,** the test compound prepared by diluting to 2X final desired concentration and serially diluted in reaction buffer. Azide solution diluted to 0.30% (2X concentration or 0.15% final 1X concentration) inhibits approximately 50% of the integrase activity. Also, in each experiment, 50 µl reaction dilution buffer negative control (assay buffer containing 10% dimethyl sulfoxide (DMSO)without any enzyme) was included. The IC50 values were determined from a curve (drug concentration versus percent inhibition) fitted on experimental data (Prism software) to obtain the concentration that produced 50% inhibition.

2.5. HIV-1 reverse transcriptase (RT) enzyme-activity assay

The reverse transcriptase assay, has been made for colorimetric method and is designed for use in research studies, as a method for the quantitative determination of RT activity in cell cultures and other biological samples. The inhibitory activity of nanofluid product against HIV-1 RT was measured using a commercial enzymatic kit - EnzChek Reverse Transcriptase Assay kit (Thermo Fisher Scientific, USA). Separately, the recombinant HIV-1 RT enzyme was provided from Worthington Biochemical Corporation (USA). So, at first, in wells of a 96-well plate, a different concentration of the nanofluid was added to certain amount of HIV-1 reverse transcriptase. Then, the mixture was applied to the above-mentioned RT assay kit according to manufacturer's protocol. Two wells were allocated to highly active antiretroviral nevirapine (nonnucleoside reverse transcriptase inhibitor) (Sigma-Aldrich) as positive controls whereas negative controls were infected wells that had no drugs or nanoparticles. Because, nanotechnology science has been considered a platform to circumvent some of the challenges in HIV/AIDS treatment.

3. Results and discussion

To date, no definitive treatment for AIDS has been found, but it is possible to prevent the progression of the disease and counteract its symptoms, mitigating the severe immunological response. In fact, there exists very effective drugs that are used to fight with this dangerous virus and its complications, via reducing the virus in the patient's body, improving the health of the immune system as much as possible and reducing the complications of the disease. An important reason why physicians fail to treat AIDS so far is that the HIV virus would remain in the patient's stem cells for a long time. Therefore, we need to develop a new drug that can reach the stem cells and separate many cells infected with AIDS from other healthy cells. In this work, we have attempted to improve and apply nanotechnology science as effective nanocomposites to introduce a big step towards the treatment, prevention and control of the AIDS virus. In this regard, ZnO Q-Dots/CNTs@Fe_3O_4 nanocomposites is a strong nanofluid with a great impact on the HIV-1, preventing from destroying the components of immune system like CD4+ T cells. It was characterized by effective methods such as SEM, TEM, EDS, Mapping of EDS, XRD, FTIR, and UV–Vis. Each of these identification methods applied could confirm the newly-born structure of nanoparticles and nanocomposites being made in nanofluids. Next, we studied the mechanism and function of the resulting synthesized nanofluid at the site of contact and the interface between nanofluid and HIV viruses.

3.1. Characterization of the new ZnO nanofluid product

3.1.1. SEM images of prepared ZnO Q-Dots nanoparticles and nanofluid product

In this part of study, you can pay close attention to four typical surfaces of morphologies that is synthesized (SEM images of a, b1, b2, and b3) in Figure 2. Afterwards, we will focus on b3 picture deeply thereafter.

Scanning electron microscopy (SEM) images of two significant surface morphologies of ZnO-Q-Dots nanoparticles and ZnO nanofluid product are shown in Figure 2 (a) and (b1-b3), respectively. The nanoscales of SEM images for these pictures are different. As can be seen, the ZnO nanoparticles are very fine and spherical quantum dots which enable such structures to have good permeability to attack layers of viruses. The presence of various NPs of iron oxide and carbon nanotubes in the nanofluid formulation is well seen in Figure 2 (b1) and (b2). These nanoparticles and nanocomposites were solved in mixture of solvent, alcohol, emulsifier, and stabilizer in nanofluid product. Hence, NPS dispersed among of these deferent fluids' layers. A part of the accumulation and presence of these NPs can be seen in the morphology layers of Figure 2 (b3). Showing how the NPs fit and sit in a selected layer of the dried nanofluid morphology is an interesting test for further mapping of EDS analysis.

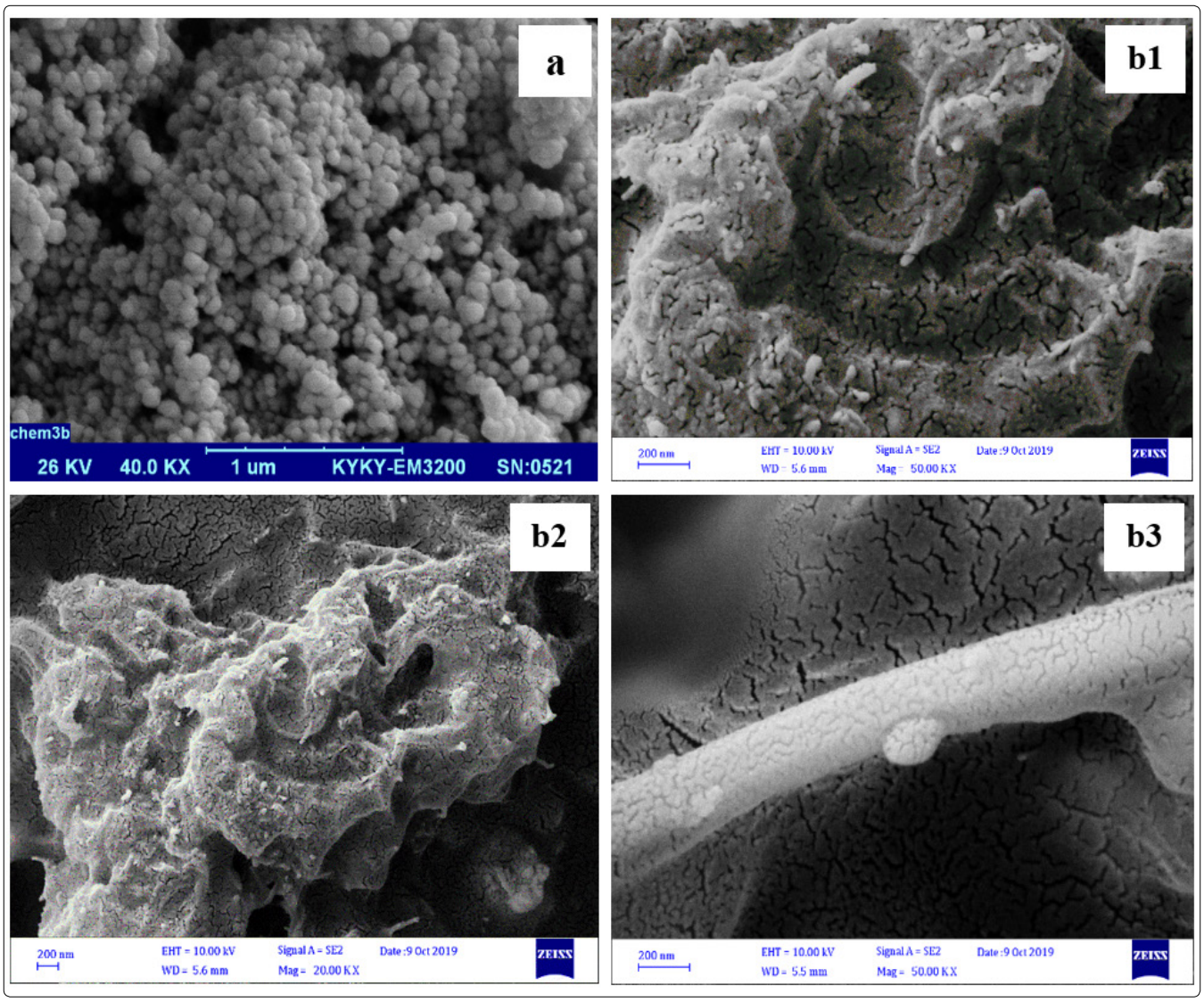

Figure 2: SEM images of (a) homogeneous nano spherical of ZnO Q-Dots NPs. and (b1, b2 and b3) the mixture of spherical and carbon nanotubes in nanofluid product, when it was dried at 150 ºC in oven without any solvent.

3.1.2. EDS analysis curve for original nanofluid product

Figure 3 is shown the EDS analysis curve for original nanofluid product. The basic metallic elements in the product is clearly visible. They are C (in CNT), Zn (in ZnO Q-Dots and ZnO@CNT/ fatty acid nanocomposites), Fe (in modified-CNT@Fe_3O_4). The wt.% for these elements are: Zn (54.3%), C (31.4%), Fe (0.9%), respectively. The intensity and height of the peaks indicates the high % weight of elements in the compound. In the following, elemental mapping of a sample and image analysis was done as well.

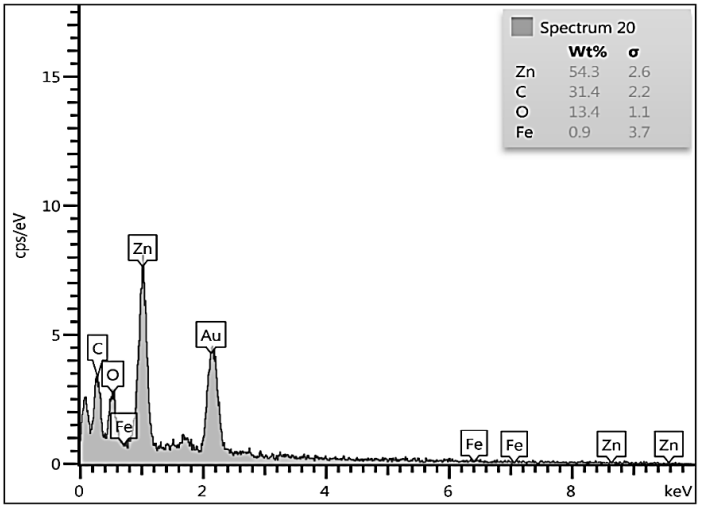

Figure 3: EDX- profile curve for original nanofluid product in dried condition.

Energy-dispersive X-ray spectroscopy (EDS or EDX) is an analytical technique used to identify the elemental composition of materials. EDX systems are attachments to Scanning Electron Microscopy (SEM) for the elemental analysis or chemical characterization of a sample. This analysis is very important to identify and present the contributing fundamental elements composition in the manufactured product, especially for knowing the percentage of their amounts in dried nanofluid. In this analysis, it is observed that the zinc metal content is highest and followed by carbon nanotube and iron oxide respectively, which corresponds with expected ratio in the synthesis conditions of the nanoparticles and nanocomposites in this work and no other extra elements were observed.

3.1.3. A morphology map (plane-view SEM images) of the original nanofluid product

Figure 4 presents the mapping of EDS layered which was previously presented in image (b3) of Figure 1. The X-ray mapping is an extremely powerful technique to understand the distribution of all the constituent elements in the nanofluid product. It demonstrates the quantitative capabilities of X-ray mapping techniques in Figures 4 and 5 for selected b3 picture. In this special layered, the various elements with different colors in 500 nm scale is shown. The total collection of nanoparticles is placed near together in part of a surface morphology of nanofluid product in dried form. They are evidences of starting materials in synthesis of various NPs employed. This method is used to separate and characterize a phase from the background phase. X-ray mapping remains the most convenient and popular method for producing "dot map" compositional images and determining elemental associations.

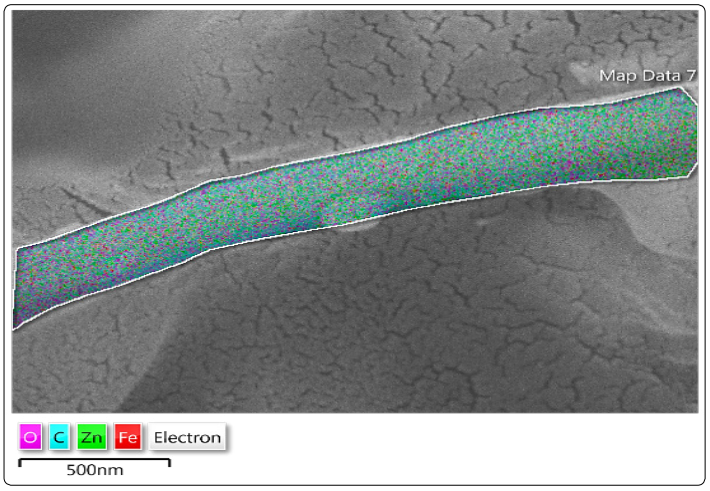

Figure 4: EDS layered electron image for homogenous distribution of the basic elemental composition of materials, confirming existence of uniform particle sizes in SEM picture of (b3).

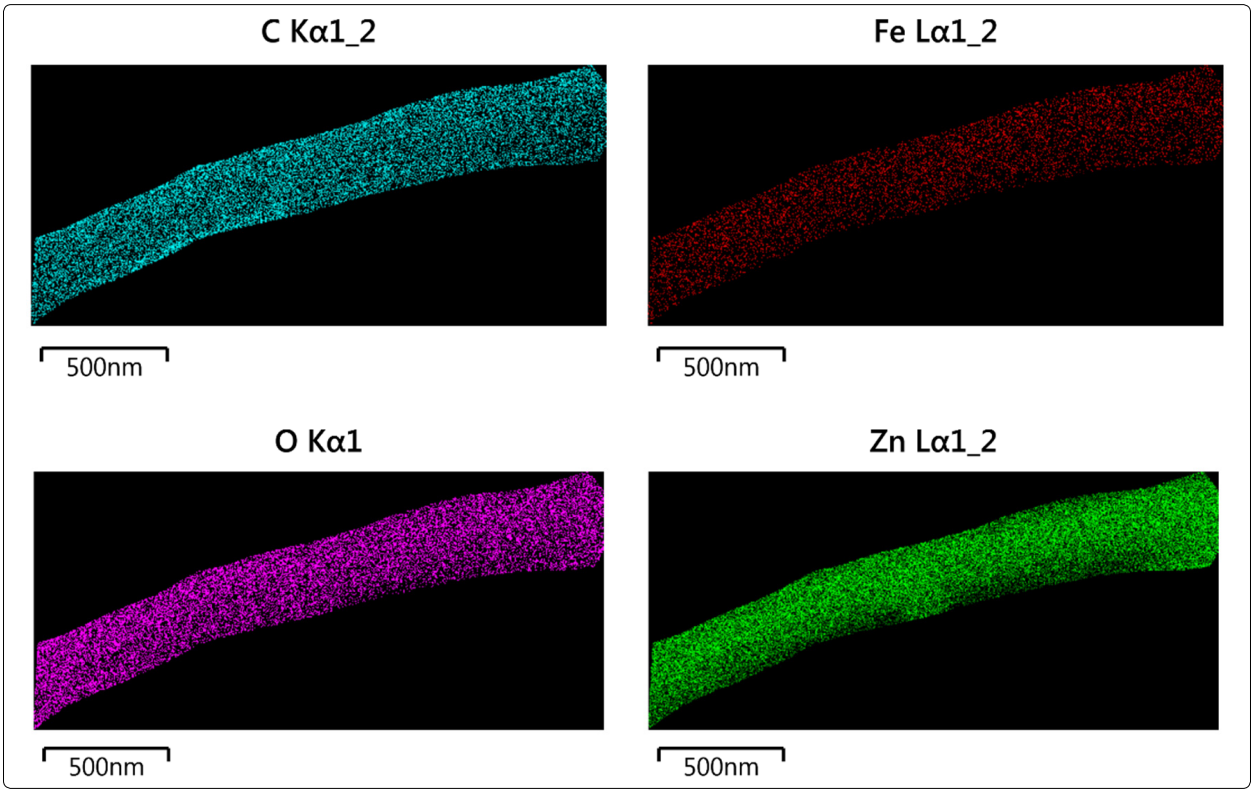

Figure 5: An interesting representation of the distribution of individual elements into the layers for the nanoproduct map. Blue color represents C, red indicates Fe, purple shows O, and green depicts Zn basic elements, respectively.

In Figure 5, we can see images of unique homogeneity distributions for C, Fe, O, and Zn selected basic elements in the Kα and Lα layers into the total X-ray mapping of the dried nanofluid product with different colors in 500 nm scale. Each color in Figure 5 contains a constituent element in the product. The amount of each element in the product can be deduced from the accumulation of

these colors in each individual segment. This accumulation of elements shows how the constituents and nanoparticles are distributed in that particular morphology image. According to the EDS analysis, the percentage of Zn-rich metal particle is much more than carbon and the percentage of oxygen is much higher than that of iron (Fe). The elements of Zn and Fe are located in the Lα1_2 layer but C is in the K α1_2 and O is in the Kα1 layer, respectively.

3.1.4. TEM microscope pictures of original nanofluid product

Transmission electron microscopy (TEM) is a substantial technique to confirm the existence of such significant nanofluid product in various nm scales. Figure 6 presents the appearance of carbon nanotubes, Fe_3O_4 and ZnO Q-Dots spherical NPs in 20, 30, and 60 nm with various magnifications (KX). Nanofluid product contain carbon nanotubes modified which have sited on the base iron oxide magnetic NPs and produce new nanocomposites CNTs@Iron oxide. On the other hands, zinc oxide@CNTs-modified could make significant nanocomposites, as well. There are also spherical zinc oxide NPs which exist separately in the nanoformulation. Taken together, all of these nanostructures in formulated nanofluids could produce interesting hybrid networks, which we can see them by TEM. As it is clear in TEM images, the black dots scattered on the carbon nanotube plates are the same as the iron oxide NPs. Herein, zinc oxide NPs are seen as white dots throughout the network. In this hybrid network, CNTs are somewhere as an activator and elsewhere as the base of nanocomposite. For this reason, CNTs are observed in higher concentrations in the images. The surface of CNTs-modified NPs causes that all components are well dissolved in polar solvent. The presence of surface-modified CNTs, magnetic iron oxide NPs and zinc oxide quantum dots NPs in the nanofluid can produce a successful oxidizer sample for treatment of HIV/ADIS.

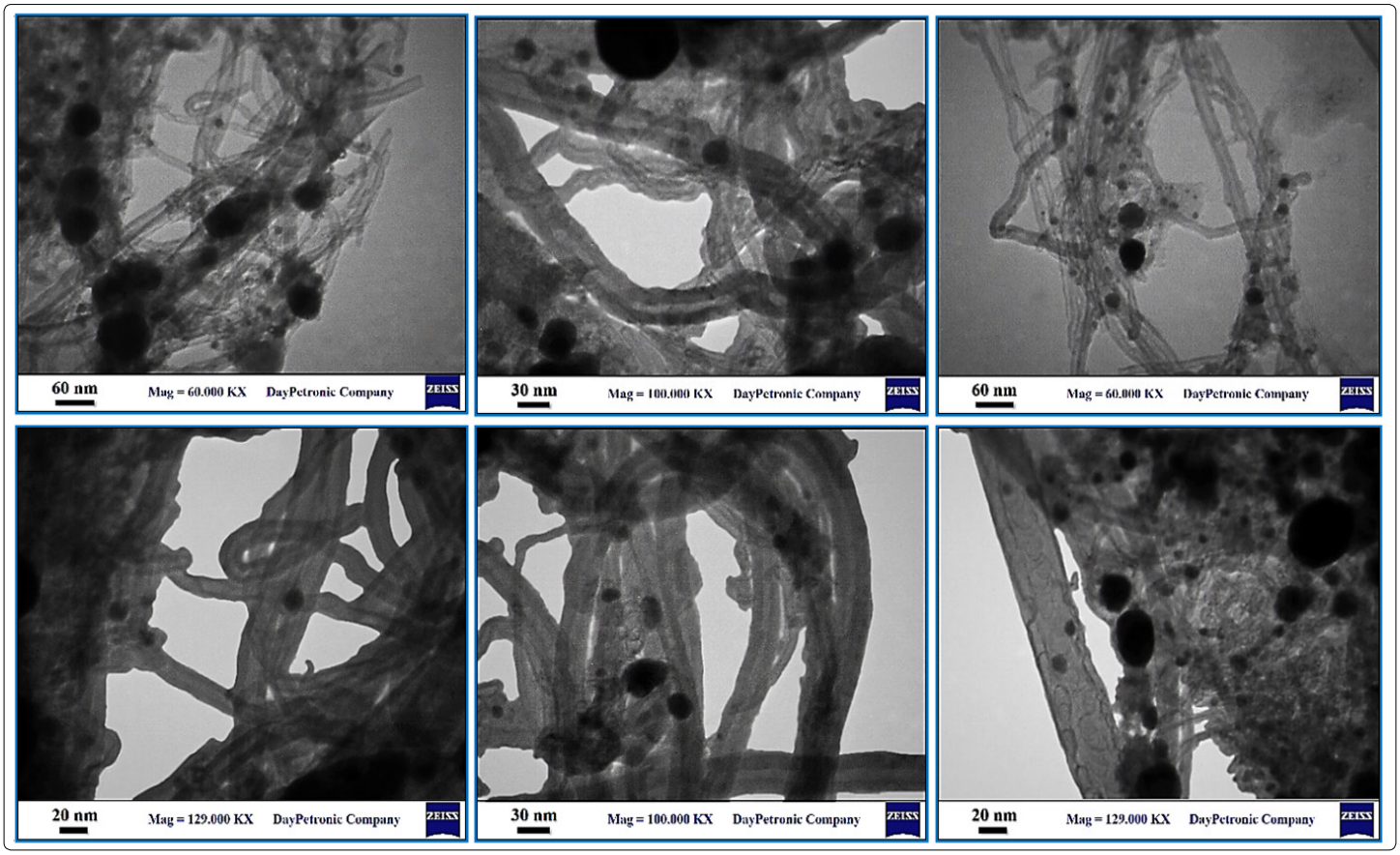

Figure 6: TEM images of mixture of spherical shape nanoparticles and multi-walled carbon nanotubes (CNTs) in water-based nanofluid product, in different 60, 30 and 20 nm scales.

3.1.5. FTIR spectra and XRD pattern of hexagonal spherical structure of ZnO Q-Dots and iron oxide magnetic among CNT NPs.

Fourier transform infrared (FTIR) spectroscopy analysis was used to determine the functional groups of ZnO/CNTs@Fe_3O_4 nanocomposites (Figure 7). FTIR results also show the absorbance peak intensity of the functional groups in sample. FTIR spectrum acquired using a Bruker Co. Model Tensor 27 spectrometer in KBr matrix. The high broad band intensity at 3425 cm^{-1} region corresponding to the vibration mode of –OH stretching of hydroxyl group in alcohol and carboxylic acid groups belonging to fatty acid in nanocomposites. Two sharp bands at 2923 cm^{-1} and 2855

cm^{-1} are assigned as carbon-hydrogen C-H symmetric and asymmetric stretching of respective vibrations. Low intensity peak –OH bending of the hydroxyl group at 1635 cm^{-1} at 1350 cm^{-1} is also visible, indicating –COOH groups and zinc carboxylate (COO–). Two absorption bands belonging to C–OH bond were observed at 1099 and 948 cm^{-1} that correspond to stretching and deformation modes. In the spectrum containing fatty acid, these peaks were observed with little displacement. According to the FTIR spectrum of ZnO and Fe_3O_4, the peaks at 772 and 560 cm^{-1} imply to Zn–O and Fe–O bonds, respectively [26,28]. Comparing the FTIR spectra of ZnO/CNT@Fe_3O_4 nanocomposites with separate ZnO quantum dots (QDs) and Fe_3O_4 NPs spectra confirms the successful production of the nanocomposites through the synthesis process. It should be noted, that the surface of carbon nanotubes (CNTs) used has been modified with two different categories of methods whose effects can be seen in spectrum a.

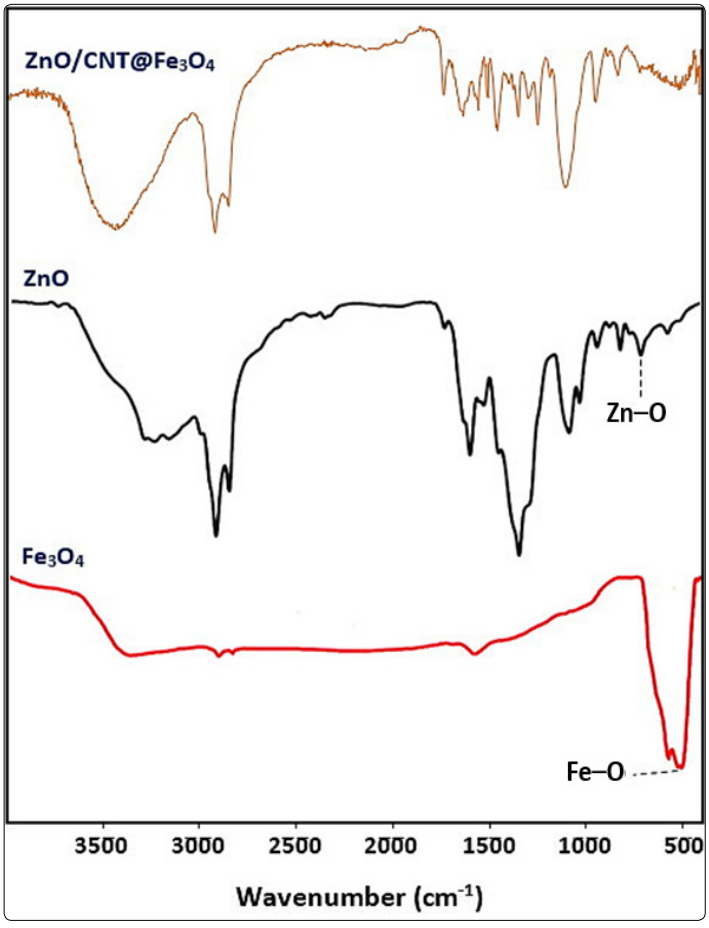

Figure 7: FTIR spectrum of the synthesized powders of ZnO/CNTs@Fe_3O_4, ZnO and Fe_3O_4 nanoparticles over 4000-400 cm^{-1} range.

The X-ray Diffraction (XRD) pattern using CuKα radiation (λ = 1.54Å) for ZnO Q-Dot NPs is illustrated in Figure 8. Because the main base of this nanodrug consists of ZnO NPs, we should be able to obtain its structure by XRD pattern. The scans were measured in the 2θ range between 10 and 90°. Strong diffraction peaks at 31°, 34°, 36°, 47°, 56°, 62° and 67° 2Θ can be attributed to the (100), (002), (101), (102), (110), (103) and (112) crystal reflection planes, respectively, of hexagonal Wurtzite structure of zinc oxide. This result is in well accordance with JCPDS standard card File No. 361451 for ZnO NPs [29].

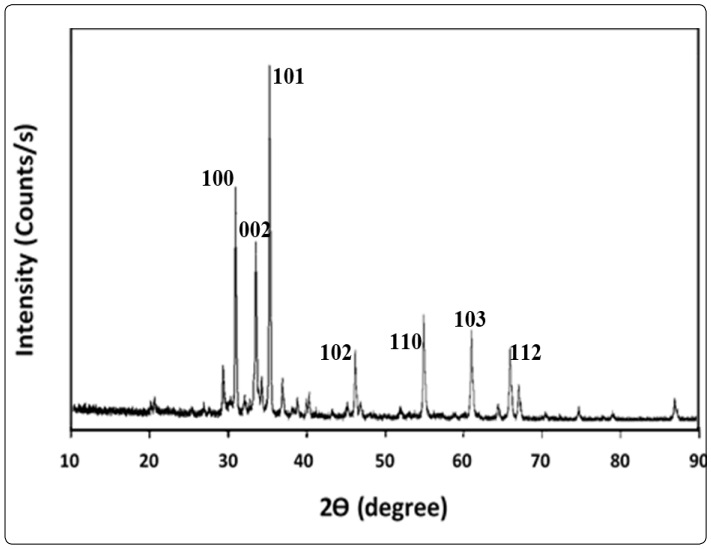

Figure 8: XRD diffraction patterns of Wurtzite ZnO Q-Dots nanospherical particles with hexagonal phase according to lattice constant of a = b = 3.249 Å and c = 5.206 Å with JCPDS standard card no. 36 – 1451 including space group p63 mc.

3.1.6. UV-Vis absorption spectroscopy of spherical structure of ZnO Q-Dots

UV–Vis spectroscopy (Perkin Elmer model lambda 35), of fine spherical ZnO Q-Dots is shown three peaks at 199, and 240 (max. absorption) and 275 nm wavelengths in Figure 9. Actually, this event can attribute to a special modified surface state of ZnO Q-Dots during its synthesis processing. UV-Vis of tiny semiconductor ZnO Q-Dots NPs containing wide band gaps energy (4.8 eV), strong inner shell electron defects (oxygen vacancies), quantum confinement effect, high surface energy, many quantized surface trapping states, and special surface area. In addition, the surface modified ZnO Q-Dots illustrates the lowest wavelengths of ultraviolet light revealing very fine porous nanomaterial. In this regard, they illustrate specific electronic (electron holes) properties (photoluminescence) in optical characterization that differ from larger particles due to quantum mechanics. With this condition, they can shift towards blue emission (blue shift). Accordingly, in advanced nanoscience and nanotechnology when the Q-Dots nanoparticles size is very small (1-4 nm) they can absorb and emit shorter ultraviolet light wavelengths yielding colors like blue and green. This is because of having high surface energy and large number of surface atoms or molecules (effect of size on optical properties, Bohr exciton radius) [25-26].

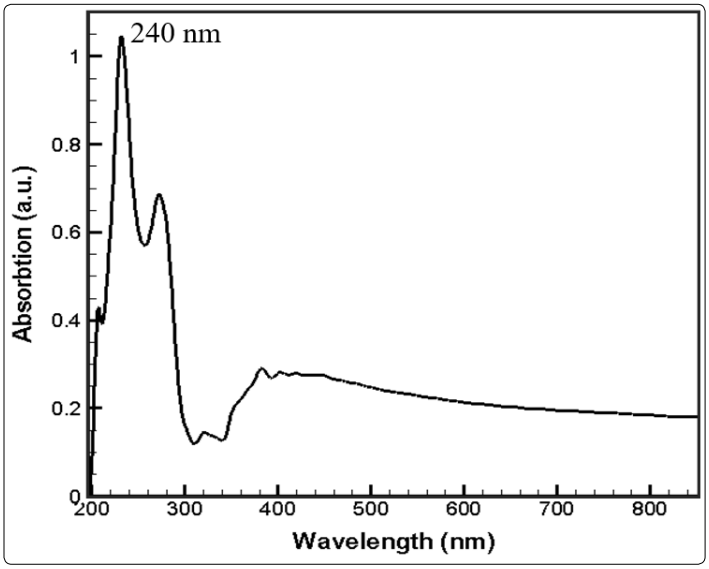

Figure 9: The plot of UV–Vis absorption spectrum of ZnO QD NPs vs nm wavelengths, as one of the most important nanofluid components.

3.2. Time-dependent manner of replication inhibition

First generation of single-cycle replication (SCR) assay of HIV-1 virions pseudotyped by glycoprotein G of vesicular stomatitis virus (VSV-G) was determined after infection in HEK 293 T (human embryonic kidney, from Pastor Institute of Iran) cells treated with a range of nanofluid product concentrations [30]. Most people infected with HIV develop specific antibodies (i.e. seroconvert). Diagnosis of primary HIV before seroconversion is done by measuring HIV-RNA or p24 antigen. In curve of Figure 10, HIV-1 p24 antigen (Ag) is plotted in time (hours), whereas the desired times chose from zero to 100 hours. The curve has been climbing from zero to 50 hours, but has declined after 50 hours.

HIV-1 p24 production was started on the first day and reached to its peak on second-day post infection. The p24 antigen is a protein that is part of the HIV structure and in the early stages of infection, it is produced in large quantities. It can be detected in the blood by diagnostic tests. Therefore, p24 antigen testing is used in early detection of HIV. However, p24 secretion was gradually decreased from the fourth day postinfection (Figure 10). As the results denote, the maximum cumulative amount of p24 in the supernatant was 72 h after inoculation. Therefore, the p24 determination in the presence of the supernatant was used to assess the viral replication at 72 h after inoculation. Certainly, the photos of the first generation SCR HIV-1 virions morphologies, in the form of infected and non-infected of syncytium formation, are clearly changing. As non-infected cells grow larger and stick together (deformation and agglomeration). HIV and opportunistic infections may be caused by bacteria, viruses, fungi, and parasites that are normally controlled by the immune system. For this purpose, we measured the inhibition ability of nanofluid product on HIV virus replication. The nanofluids were applied at 0.1, 1, 2, 5, 10, 50 and 100 µM concentrations on infected Hela cells and the EC50 (concentration of a drug that gives half-maximal response (value of nanofluid product was determined to be 2 µM, using non-linear regression analysis from transformed data (Figure 11 & Table 1). In Figure 11, SCR HIV replication inhibition (%) is plotted in concentration of nanofluid product in order to the identification and analysis of HIV inhibitor. This curve shows an upward trend in terms of increasing SCR HIV replication inhibition values as well as increasing logarithm of nanofluid product concentration on the horizontal axis. The HIV-1 SCR has a better biological safety and may be a promising candidate for the vaccine studies in future. SCR HIV p24 antigen (Ag) created in this research are capable of one cycle of replication and will be inactivated afterward.

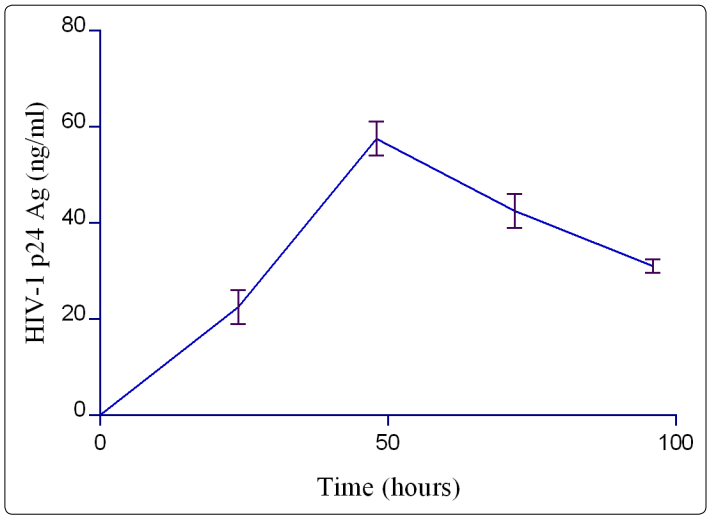

Figure 10: The Single cycle replicable (SCR) HIV-1 p24 antigen (Ag) production levels at different time post inoculation in Hela cell line.

In Figure 10, the HIV-1 p24 antigen (Ag) concentration curve was plotted against the time (h). As we can see, in the first 50 hours, its concentration was calculated below 50 ng/ml for Hela cell line. A good result for Hela cell line has been obtained from this experiment in vitro.

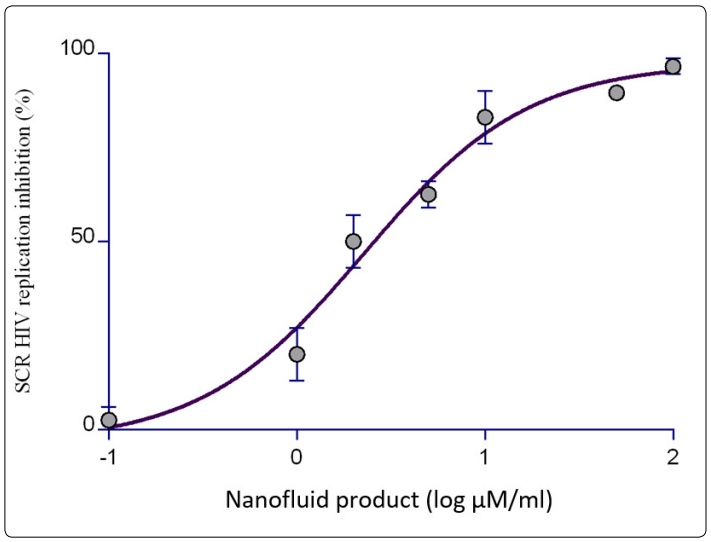

Figure 11: The effects of nanofluid product on the HIV-1 replication at different log µM/ml concentration exposures.

From Figure 11 curve it can be seen that the system is based on the replication assay SCR HIV-1 in terms of logarithm of concentration and measuring the rate of replication of SCR virions. Herein, the SCR HIV-1 replication assay system significantly shows the antiviral effect of this nanodrug at different concentrations. From these data, it is easy to calculate the antiviral activity of this nanodrug at different concentrations. As the SCR HIV-1 replication inhibition factor increases, the slope of the nanodrug concentration curve increases and the potency and ability of this nanodrug in preventing AIDS is developing. From this curve and these findings, it can be concluded that this new antiretroviral nanodrug is effective in inhibitory of AIDS and works very well and it will place among notable results obtained by other scientists, till now and beyond.

3.3. Characterization of the mechanism of HIV-1 replication inhibition by nanofluid product

Nanofluid product was tested in vitro for its ability to inhibit the HIV-1 reverse transcriptase. IC50 values were generated from duplicate experiments and calculated using dose-response curves (Table 1).

Compound	HIV-1 Replication Assay (EC50, µM)	RT Assay (IC50, µM)	Integrase assay (IC50, µM)	Cytotoxicity (CC50, µM)	TI = CC50/EC50[d)]
Nanofluid product	2	5	15	25	12.5
Zidovudine	0.03	0.02	N.T.	>200	
Raltegravir	0.04	N.T.	0.01	>200	

Table 1: Anti-HIV-1 replication activities (EC50 values), inhibitory activities (IC50 values) towards HIV-1 reverse transcriptase and integrase activities, and Cytotoxicities (CC50 Values, XTT Assay) of the nanofluid product. (N.T. refers to not tested) and (d) refers to dose-response.

HIV is a complex virus. These cells are mainly produced in immune-specific cells called CD4 lymphocytes. CD4 cells are white blood cells that play an important role in the body's immune system and the body's natural defense against pathogens, infections, and diseases. During HIV replication, CD4 cells are destroyed and as the cells get more and more killed, the body loses the ability to fight off many infections. Antiviral screens have shown for the identification of novel (HIV-1) inhibitors. In this work, we investigated an HIV-1 replication assay that include all of the targets required for replication in T-cell lines. HIV-1 Rep assay (2 µM), quantitative determination of retroviral reverse transcriptase activity (RT assay, 5 µM) were evaluated in Table1. Moreover, for Raltegravir controller, RT Assay did not test. Because, Raltegravir is specific as viral integrase so it does not affect reverse transcriptase and is not tested. Reverse transcriptase enzymes are enzymes capable of DNA synthesis by RNA. It is clear that the higher the RT factor, the higher the CD4 lymphocyte counts, and elongate survival in HIV-1-infected patients and finally reduce the incidence of opportunistic infections. Integrase also plays a crucial role in the HIV-1 life cycle coordinating the integration of the reverse-transcribed viral DNA into the host genome. In vitro HIV-1 integrase enzyme assays (15 µM) was obtained and cytotoxicity (TOX) concentration 50% (CC_{50}) of antiretroviral drug (25 µM) was also compared with two well-known Zidovudine (ZDV) and Raltegravir (RAL) as controlled clinical trials and references. Zidovudine is an anti-reverse transcriptase drug, hence, integrase testing has not been performed in Table 1. CC50 concentration value provided evaluable information on the concentration of nanodrug that will kill half the cells in an uninfected cell culture. In addition, the nanodrugs with CC50 values of greater than 5 micromolar (µM) are referred inactive. TI (Therapeutic Index) of medicine is a quantitative measurement of the relative safety of nanodrug and it is the ratio of the dose that produces toxicity to the dose needed to generate the desired therapeutic response (the relationship between efficacy (pharmacology) and safety (toxicology)). Anti-HIV activity was assessed by the ratio of CC50 value to the concentration towards 50% cytoprotection from HIV infection (EC50). TI=CC50/ $EC50^{d}$ (toxic dose 50/effective dose 50) was achieved as 12.5 for this nanofluid product in this study. The larger the therapeutic index (TI) the safer the nanofluid product regarding to having favorable safety and efficacy profile. TI index should not be very small amount, and the difference between the blood concentration at which a nanodrug becomes toxic and the concentration at which the nanodrug becomes effective should be suitable. From these calculations, it has been found that the synthesized nanofluid product as a nanodrug candidate is superior to the control drugs and showing its potential for therapeutic application and can slow down the progress of the virus and disease and maintain the immune system. Because the TI factor represents an important indicator of the probability of the successful development. This work can be expanded by doing in vivo special animal model tests and then presenting it to the pharmaceutical industry as a new drug for treatment and prevention of AIDS (HIV-1). As a suggested mechanism, NPs are able to bind with protein's organic amino acids through N-terminal, C-terminal and catalytic core domains and generate enzyme active sites which causes oxidation chemical processing. These bonds can establish electrostatic interaction with –S and –N donor elements in enzymatic reactions, which behave similar with a nucleophile site. In the other hand, Van der Waals contacts and hydrogen bonds reactions can demonstrate significant role in direct biochemical method. Coordination complexes and organometallic derivatives could mediate in producing auxiliary ligands and anionic O-donor groups. Oxidizing agent like Fe_3O_4 (Fe^{3+}) NPs found to be in a six-coordinate environment with oxygen-rich ligands. The aromatic surfaces of carbon nanotubes (CNTs) NPs are able to interact chemically with virus proteins and induce the oxidation of cysteine residues of the proteins through holes, van der Waals, hydrophobic, π–π electron interactions. Actually, their hexagonal surfaces of CNTs are coated by protein layers which can affect the redox reactions of the CNTs and their companions herein [31]. They can

produce charge transfer from the sulfhydryl groups (R-SH) of the cysteine residues and make disulfide (sulfur-sulfur) bonds which are very important in biological systems. The interconversion between thiols and disulfide groups is a redox reaction: the thiol is the reduced state, and the disulfide is the oxidized state (thiol/disulfide redox state). The CNTs NPs used in these nanocomposites were modified in two different forms. 1- with –OH group as an oxidizer nanoagent, 2- with –COOH group as a reducing nanoagents. That's why they have the power of redox reactions together in chemical oxidation-reduction reaction. In fact, CNTs NPs and their nanocomposite hybrids could cause protein oxidation, using the O_2, H^+ / H_2O couple receive electrons by oxidizing thiol groups (S-H) such as amino acid cysteine and methionine (CNT– protein or CNT– thiol interactions). The reducing part of the nanopackage contains –COOH-CNTs, which has the power to fight with HIV envelope glycoprotein and reduce them and destroy harmful GP120 molecule of HIV virus. Conducting/adapting/controlling redox reactions of sulfhydryl groups of protein cysteine and methionine residues with our new nanofluid containing oxidizing part of nanopackage formulation regarding to treatment of HIV-1 presents a new idea for the first time. To fight viruses with the structure of HIV-1 and Covid-19 and even cancers that are caused by viruses, complex and logical nanofluid manufacturing technologies must be used [32].

4. Conclusion

The composition of ZnO Q-Dots, CNTs@Fe_3O_4 nanocomposites and ZnO@CNTs as a strong nanofluid could control, treat and suppress HIV infection (AIDS) type-1 well. Therefore, this significant synthesized nanofluid can be a good promise for nanomedicine and nanobiotechnology as antiretroviral medication and anti-HIV agent. Undoubtedly, this nanoproduct is able to prevent immune system disruption by connecting to important protease enzyme, modifying HIV-1 reverse transcriptase (RT) and integrase activity. Also, it has this smart capability to potentially run the oxidation of sulfur containing amino acids such as in cysteine (Cys) and methionine (Met) residues via producing reactive oxygen species (ROS). IC (50)'s ranging from 2, 5, 15 and 25 µM were obtained for HIV-1 replication assay, RT assay, integrase assay and finally cytotoxicity respectively, compared with Zidovudine and Raltegravir according to Figures 10, 11 and Table 1.

In this work, the nanoproduct package indicated potent oxidizers of HIV-1 RT and integrase enzymes due to the presence of effective nano-oxidizer and nano-reductant materials in the nanofluid formulation. In this regard, the fine synthesized ZnO Q-Dots NPs were able to diffuse into layer of HIV virus proteins. Other co-activators such as modified oxidant-CNTs /Fe_3O_4 and ZnO/modified reductant-CNTs in nanofluid package which could provide synergism effects and act as strong oxidizing and reducing agents to inhibit both RT and protease enzyme activity. In this study, modified-CNTs@Fe_3O_4 and ZnO@modified-CNTs nanocomposite hybrids could present (in Table 1) redox reactions simultaneously, suppressing the intermolecular association of Cys and Met proteins (containing free thiol groups) as they exhibited a remarkable synergistic effect together by strong adsorption of virus proteins onto their active interface of nanosurfaces. An important conclusion obtained from this research is that the synthetic nanofluid product has two different properties. It has both oxidizing and reducing nano groups. Each of which carries out its own mission. This is a new idea for making a nanodrug for HIV infection or other viruses like Covid-19 that comes from a chemist's logic.

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