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Characterization and DFT Studies for Green Synthesis of Silver Nanoparticles by Morphine Ampules and their Anti-proliferation Activity

Amineh Shafaei[1], Gholam Reza Khayati*[,2], Reyhane Hoshyar[3]

[1]Department of Nanotechnology, Mineral Industries Research Center (MIRC), Shahid Bahonar University of Kerman, Kerman, Iran.
[2]Department of Materials Science and Engineering, Faculty of Engineering, Shahid Bahonar University of Kerman, Kerman, Iran.
[3]Cellular and Molecular Research Center, Department of Biochemistry, Faculty of Medicine, Birjand University of Medical Sciences, Birjand, Iran.

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** Corresponding author email: khayati@uk.ac.ir*

ABSTRACT

This study is a green approach for the synthesis of silver nanoparticles (AgNPs) using morphine ampoules (MO) as reducing and capping agents. The toxicity effects of prepared particles were evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on breast cancer cells. UV–visible spectrum, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction analysis (XRD), environmental scanning electron microscopy (ESEM), transmission electron microscopy (TEM), and dynamic light scattering (DLS) techniques were employed for characterization of prepared particles. A review mechanism has been done based on the density functional theory (DFT). The results confirmed the formation of spherical and crystalline AgNPs with the average particle size of 50nm. Also, DFT analysis revealed that the reduction was occurred by connection of MO constituents to the NO_3 part of $AgNO_3$. In addition of reducing effect, the capping characteristics of MO biomolecules were proved by FTIR spectra. Moreover, comparison of Anti-proliferation Activity of MO-AgNPs was 69% higher than MO at similar dosage. Therefore, in addition to use MO as a painkiller in the treatment of cancer, it can also be used as a factor for the synthesis of AgNPs with enhanced its anticancer properties.

Keywords: *Green synthesis; Morphine ampoules; Breast cancer cells; Density functional theory; Biomaterials; Nanoparticles.*

1. Introduction

Nanomedicine potentially has a major impact on the human health soon. Nanoparticles such as silver nanoparticles (AgNPs) have highlight biological properties and recognized as strong anti-proliferation agents with the significant therapeutic efficacy [1-4]. In general, there are various physical and chemical methods such as chemical regeneration, photochemical regeneration, ultraviolet, microwave, and laser radiation for the synthesis of silver nanoparticles. Chemical methods do not work well due to the formation of toxic chemical compounds, and compared to other approaches, environmentally friendly biological methods are preferred for the synthesis of silver nanoparticles [5-7].

Cancer is a multifaceted genetic disease that environmental factors play an important role in, and combinations of the different approaches have been employed for its treatment. The behavior of

each cancer varies by type and does not have the same trend. Breast cancer is recognized as one of the most common cancers in women [8]. Regardless of the type of cancer and the inherent problems on the body, undesirable pain is the common cause of various types of cancers. This unpleasant pain results in the greater psychological pressure on the patient, which also affects treatment outcomes. In this regard, decreasing the cancer pains, e.g., using opiates is of particular importance and commonly considered as one of the steps of cancer treatment. MO is a narcotic drug with significant application in cancer therapy. Also, MO has an analgesic effect and can be changed or stopped the progression of tumors [9, 10]. In previous studies [11], with the help of the green synthesis, silver nanoparticles have been obtained using of P. somniferum, i.e., as one of the most important painkillers available in traditional medicine, without any further investigation on its biological effect. To the best of our knowledge, the usage of the painkillers in modern medicine as a reducing agent for the preparation of AgNPs hasn't been reported. As a consequence, the main research highlights of the current study are (i) preparation and characterization of MO-AgNPs by UV–Visible spectroscopy, XRD, ESEM, TEM, EDS and, DLS, (ii) evaluation of cytotoxic activities of MO-AgNPs on breast cancer cells (MDA-MB-468 cells), (iii) usage of density functional theory (DFT) and FTIR study for determination of the reduction mechanism.

2. Materials and methods

An aqueous solution of $AgNO_3$ (1mM) was prepared and used for the synthesis of AgNPs. Briefly, 10mL of MO with the feed rate of 9mL/min was added to 90mL of 1mM $AgNO_3$ solution, stirred at 40°C with 130rpm for 30min. Then, the AgNPs were obtained after three times centrifugation for 20min at 15000rpm. Formation of AgNPs was confirmed by UV-Visible spectrophotometer (Scan Drop, Analytic Jena Co, Germany) at 300-700nm. The morphology and size of the MO-AgNPs were studied by ESEM (FEI-ESEM QUANTA 200, USA) and TEM (PHILPS EM-208S). FTIR was determined using BRUKER model TENSOR 27, Germany) technique. XRD patterns provided by X'pert MPD system of Philips instrument by Cu-K_α to confirm the average particle size of green synthesis of AgNPs. Particle size distribution was estimated with dynamic light scattering (DLS, ZEN 3600 model) analyzer. Point chemical analysis was performed by energy-dispersive X-ray (EDS).

Human breast cancer (MDA-MB-468 cell line; IBRC C10095) and Human normal cells (MCF10A, A-375) were purchased from Iranian Biological Resource Center (IBRC, Tehran, Iran). MTT prepared from Sigma-Aldrich (St. Louis, MO). Gibco (Invitrogen, NY, USA) provided Fetal Bovine Serum (FBS), Trypsin, Penicillin-Streptomycin, and RPMI 1640 solutions. The cells were cultured in RPMI 1640 medium (Inoclon, Iran), provided by combination of by 100 units/mL penicillin, 100mg/mL streptomycin and 10% FBS serum, after the growth at 37°C and the environment including 5% CO_2 [12]. At following, various concentrations of MO–AgNPs (0–100mg/mL) and MO were treated at different times (0–48h). Absorbance values were used as criteria for the estimation of cell viability. If IC_{50} defined as the concentration of drug that decreased the absorbance of treated cells by 50% respect to the untreated cells, it was possible to use IC_{50} as criterion for comparison of the behaviors of MO-AgNPs and MO against the MDA-MB-468 cells after 48h. Standard deviation used to express the accuracy of measured values.

The geometry optimization of the MO species was performed using CP2K quantum chemistry package [13]. The partial density of states (PDOS) of all optimized geometries were obtained to define each atom type contribution in the energy levels. The energy of highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO), the energy gap (or frontier orbitals that defined as the energy difference between the energies of HOMO and LUMO) selected for the investigation of the mechanism of reduction by MO. The molecular orbitals were visualized using VESTA software (version 3) [14]. A one-way analysis of variance (ANOVA) with the software SPSS 15 for Windows (SPSS Inc., Chicago) was utilized for statistical assessment. Each trial repeated 3 times and mean ±SD utilized to express the results. If the value of p is lower than 0.05, and then it is considered as significant.

3. Results and discussion

3.1. Characterization of MO-AgNPs

The formation of AgNPs is associated with the change in the color from colorless silver nitrate solution to the pale yellow. UV-Vis spectroscopy analysis was used to monitor the formation of AgNPs due to their surface plasmon resonance effects. In this study, the conversion of Ag^+ ions

to Ag^0 by the active molecules of MO caused the evolution of high-intensity peak at about 410nm (Fig. 1(a)). These results are in good agreement with the previous works about the preparation of AgNPs by intense absorption peaks at wavelengths in the range of 400-470nm [15]. The XRD spectra of AgNPs (Fig. 1(b)), clearly confirmed the presence of main peaks of Ag [16] as crystalline phase. Noisy pattern of XRD was the other observation in Fig. 1(b) and may be related to the crystallization of organic compounds of MO through the reduction process [17].

The surface morphology of AgNPs synthesized using MO is clearly shown in ESEM images. The nanoparticles synthesized using MO is almost spherical with wide size range distribution from 10 to be about 80nm. It seems that the constituents of MO have various reducing ability that caused to an extensive distribution in prepared particles. The results of the ESEM and EDX analysis are shown in Figs. 1(c and d). The presence of elemental silver using EDX analysis in support of XRD results is visible in the graph. Such observations indicated that the preparation of AgNPs was possible by MO. The presence of other elements including C, N and O in EDX spectrum can be related to the organic compounds of MO that attached at the surface of AgNPs [18]. The reason of the presence of higher O content as sharp peak can be related to absorbed humidity from atmosphere through EDX analysis [19]. TEM image of the AgNPs in Fig. 1(e) confirmed the formation of AgNPs with an average of 50nm. Capping of AgNPs with a thin layer, i.e., the MO biomolecules, can be observed

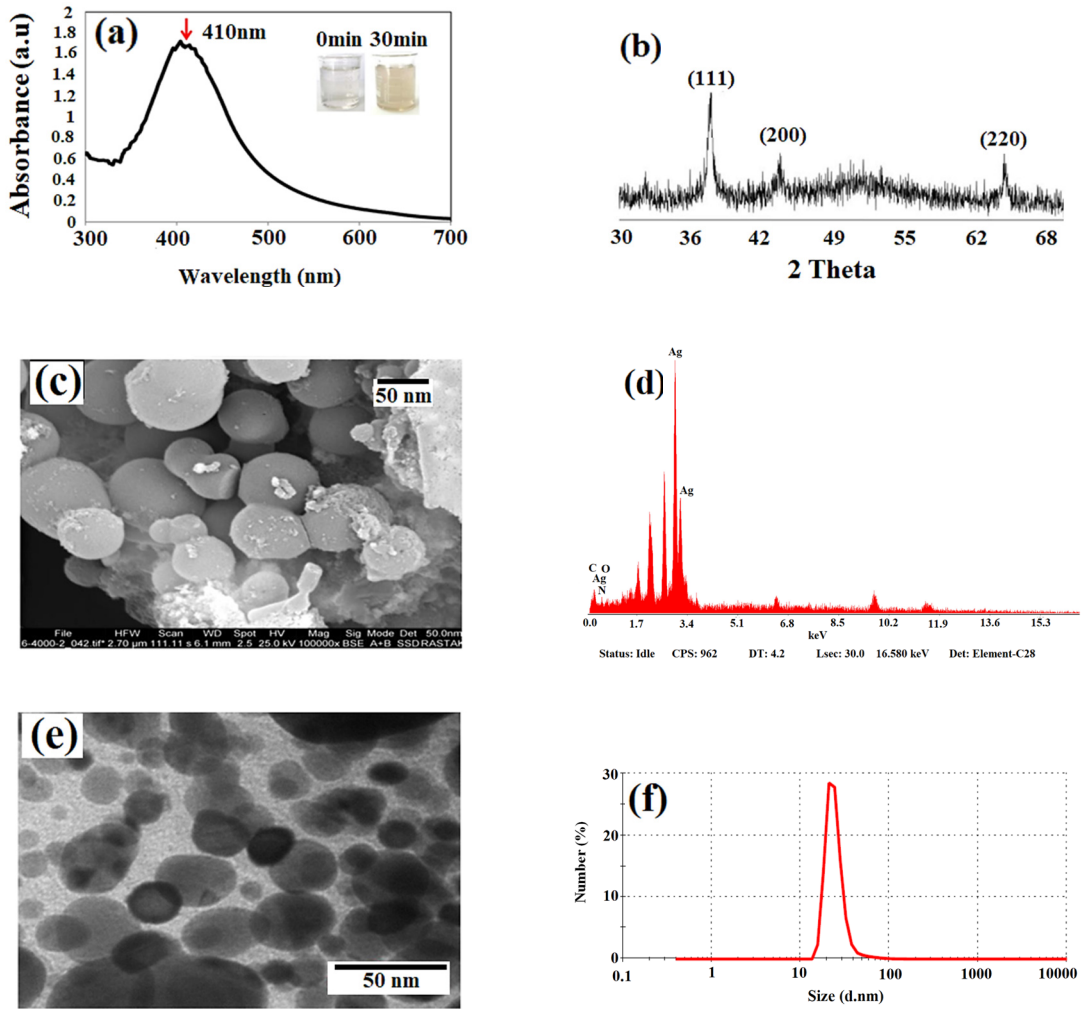

Fig. 1- (a) UV-Visible, (b) XRD, (c) ESEM, (d) EDS spectrum (e) TEM image and (f) DLS of the synthesis of AgNPs using MO.

by the lighter shadow around of prepared particles in TEM image [17]. Such observations confirmed the multiple role of MO including the reducing, stabilizing and capping agents, simultaneously.

The results of DLS analysis showed that the average size of the prepared particles is approximately to be about 20-50nm (Fig. 1(f)).

FTIR analysis used to identify the main constituent/s of that is responsible for biosynthesized of AgNPs. The peaks in the FTIR spectrum of MO and silver nanoparticles (Fig. 2) were observed at 3470 and 3433 due to O-H stretching and related to the hydroxyl functional group of phenols or alcohol [11], 2077, 2063 and 2985 due to C-H stretching vibrations aromatic compound [12], 1640, 1643,1401,1400 were due to C-C stretching of MO [13], 1095 and 1028 are due to C-O stretching [14], 649 and 662) attributed to the ring torsion modes for MO [13]. The presence of these functional groups confirmed the absorption of MO components at the surface of AgNPs.

3.2. Short Term in Vitro Cytotoxicity Assay

Recently, many researchers showed that nanoparticles have specific effects on the different diseases such as cancer. These particles can inhibit the growth of cancer cells by different mechanisms including suppressing of proliferation and cell cycle or apoptosis induction [15, 16]. MTT assay employed to study the effect of our prepared particles on the proliferation of breast (MDA-MB-468) and normal (MCF10) breast cancer cells. The results of cell assay according to the colorimetric method are presented (Fig. 3).

As revealed, that any increase in MO-AgNPs content is reciprocal to the cell viability percentage and clearly shows a dose dependent cytotoxicity activity mediated biosynthesized MO-AgNPs. The

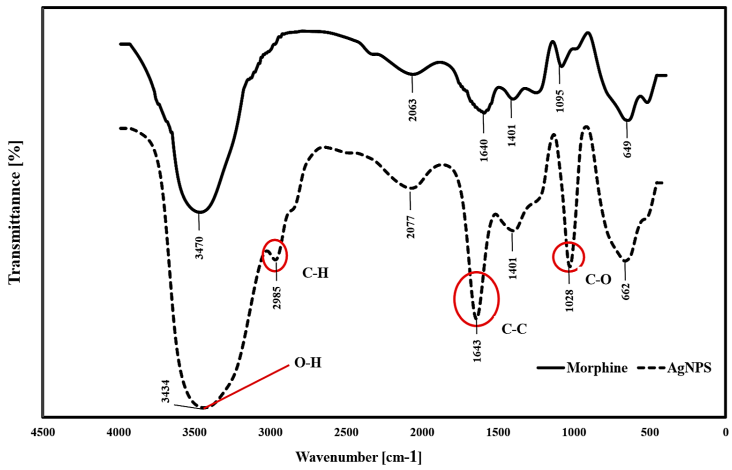

Fig. 2- FTIR spectrum for MO and AgNPs.

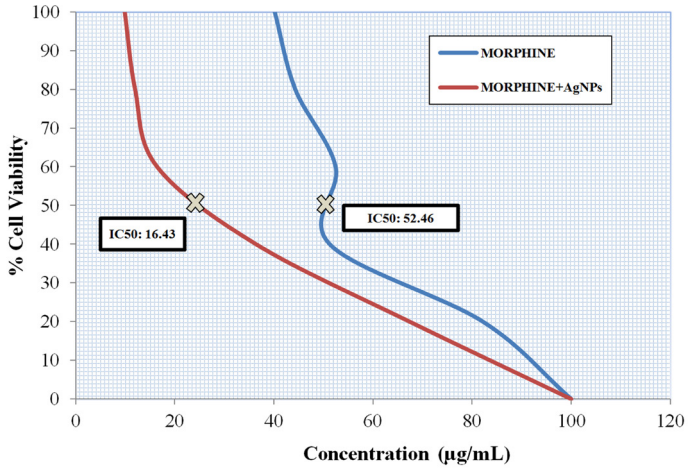

Fig. 3- MTT assay showing the cytotoxic effect of morphine ampules, (morphine+AgNPs) on human breast cancer.

IC_{50} value of MO and MO-AgNPs were detected to be about 52.46 and 16.43 µg/mL after 48 hr of treatment, respectively. The higher toxicity effect of AgNPs in the study could be attributed to their size, shape, particle surface as well as intriguing physicochemical properties. This is three times less than the effect of MO at the same concentration. Also, the colloidal MO-AgNPs showed greater cytotoxicity even at relatively low doses compared to MO. The results of these nanoparticles can be a promising way to treat human breast cancer cells.

3.3. Quantum chemical study for reduction of AgNPs

The optimized structure of the materials along with their HOMO and LUMO isosurfaces are depicted in Fig. 4(a). The reaction mechanism can be explained by means of molecular orbital theory. The reaction between two molecules takes place if the electronic repulsion by charge attraction or orbital overlap is overcome by the amount of activation energy [20]. This energy is defined by the minimum difference of energy between the HOMO energy of a molecule with the LUMO energy of the other. The orbital overlap is a necessary condition for a reaction to happen [17] and the closer the HOMO-LUMO energy of the two molecules, the faster a reaction can start. In a green synthesis of AgNPs by MO, the minimum energy difference of the HOMO-LUMO of $AgNO_3$ and the other constituents of MO are defined (Fig. 4(b)).

The diagram shows that the HOMO of MO is closer in energy with the LUMO of $AgNO_3$. Also, this amount of energy is about 0.55eV which shows that the required energy to start the Ag reduction is small. The minimum amount of energy to start the Ag reduction through the reaction of $AgNO_3$ and MO molecule is equivalent to the energy of an electromagnetic wave with the wavelength of about 2254nm that lies in the infrared realm, showing the reduction can be performed even in front of sunlight with the higher energy than infrared waves. To understand which atoms are contributed in the $AgNO_3$ reaction with MO one can obtain the partial density of states (PDOS) of HOMO and LUMO energy levels as shown for each molecule in Table 1. As explained above, the interacting orbitals of the reaction between $AgNO_3$

Table 1- PDOS* of each molecule for their HOMO** and LUMO***

Molecule	Energy Level	Ag (%)	O (%)	N (%)	C (%)	H (%)
Morphine (MO)	HOMO	-	1.85	54.06	32.27	11.82
	LUMO	-	2.84	0.29	86.36	10.51
$AgNO_3$	HOMO	84.40	15.43	0.17	-	-
	LUMO	1.49	86.84	11.67	-	-

*PDOS: Partial density of states; **HOMO: Highest occupied molecular orbital; ***LUMO: Lowest unoccupied molecular orbital.

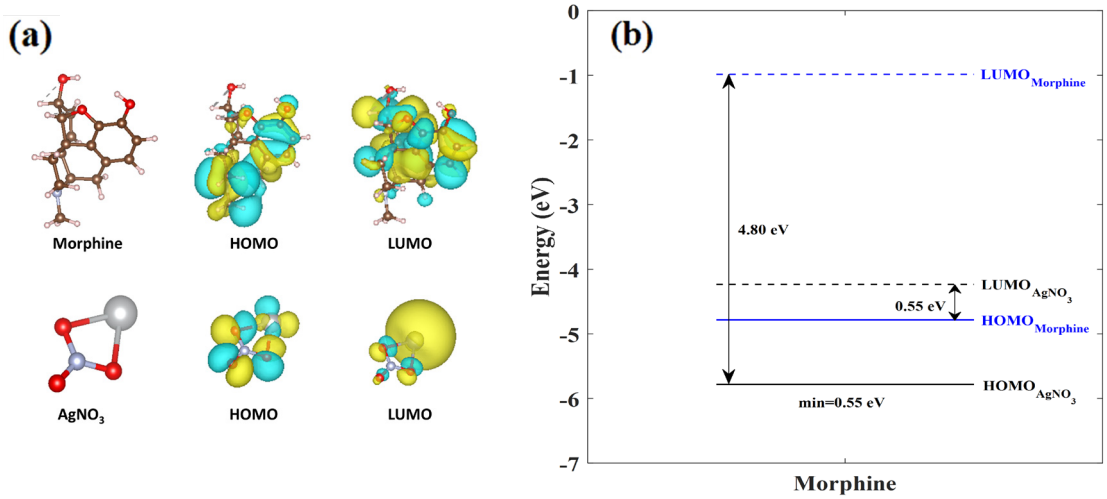

Fig. 4- (a) The optimized structure, HOMO, LUMO and energy gap of the MO; (b) The HOMO and LUMO energy levels of $AgNO_3$ (black lines) and MO (blue lines).

and MO constituents were the HOMO of MO and LUMO of $AgNO_3$. Table 1 shows that the LUMO of $AgNO_3$ is mainly composed of an oxygen atom with a contribution of about 87% and the main contributor of the HOMO of MO is N and C atoms with about 54% and 32%, respectively. When MO HOMO comes near to $AgNO_3$ LUMO, the oxygen atoms in $AgNO_3$ form bond with the C and N atoms in solution meaning that NO_3 part of $AgNO_3$ is absorbed by MO and Ag is departed.

4. Conclusion

We report a simple, rapid, and efficient preparation of AgNPs from MO. Silver nanoparticles were mainly synthesized in a 50nm spherical shape with a completely green and environmentally friendly and simple approach. These prepared particles were characterized by TEM, ESEM, EDS, DLS, UV-Vis, FTIR, and XRD. The reaction mixture absorption peak at 410nm represented the reaction mixture absorption peak at the biological synthesis range of AgNPs. Capping ability of MO constituents through the reduction was the other results of this study and confirmed by TEM image and FTIR spectrum. The synthesized silver nanoparticles showed a good anti-proliferation activity against the growth of human breast cancer cells as a dose-dependent manner. Compared to the IC_{50} value of MO (52.46 µg/mL), synthesized AgNPs were able to inhibit the growth of cancer cells in silver nanoparticles at significantly lower concentrations (16.43 µg/mL). In other words, the effect of MO was relatively 69 % lower than the effect of colloidal MO-AgNPs at the same concentration. Detailed studies based on DFT proposed the mechanism of the reduction of Ag^+ through the reaction of MO components by NO^-_3. In summery, adding to use the analgesic effect of MO as part of the treatment of various cancers, it is also possible to use it as a reducing agent for green synthesis of silver nanoparticles and enhanced the inherent anti-proliferation properties of silver, simultaneously.

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