Green biosynthesis of magnetic iron oxide nanoparticle using *crocus sativus* L. petal hydro-alcoholic extract

Hamid Mollania*[1], Majid Oloomi Buygi[2]

[1]PhD Student, Department of Electrical Engineering, Faculty of Engineering, Ferdowsi University of Mashhad, Mashhad, Iran. hamid.mollania69@gmail.com
[2]Associate Professor, Department of Electrical Engineering, Faculty of Engineering, Ferdowsi University of Mashhad, Mashhad, Iran. m.oloomi@um.ac.ir

Abstract
Nanotechnology has attracted a great interest in recent years due to its unexpected impact on many areas of science and life, especially in engineering, biology and biomedicine. Biologically synthesis of magnetic iron oxide nanoparticles (MIONPs) are among attractive nanomaterials. MIONPs have attracted the attention of many investigators owing to their magnetic properties and their ability of surface modification and high biocompatibility. Since biosynthesis is simple, cost-effective, and environmentally-friendly, it is of paramount importance in comparison to chemical and physical techniques of nanoparticles production. In the current research work, MIONPs are synthesized using a *crocus sativus L.* petal hydro-alcoholic extract. Created nanoparticles were characterized using FE-SEM, EDS and DLS methods.

Key words: *Crocus sativus L.* petal hydro-alcoholic extract, Nanoparticle green biosynthesis, Magnetic Iron oxide nanoparticles (MIONPs).

Introduction
In the nanobiotechnology, the reduction of particle size to nano extent has a significant effect on all physical and chemical properties of materials. Nano-sized particles as the base and starting point of nanotechnology are required in all research of this area; therefore, their production and synthesis is of paramount importance. Nanoparticles green synthesis has been investigated and admired in different research work as the by-product of bacterial enzymatic activities (Faramarzi and Sadighi, 2013), by the enzymatic reaction and by various plant extract. This method enjoys considerable benefits in comparison to other physical and chemical methods such as hydrothermal methods, co-precipitation, sol-gel and vapor-solid growth techniques (Kharissova et al., 2013). In fact, it is through these diverse mechanisms such as oxidation, reduction, sorption, and chelation that nanoparticles are formed and grown in an intracellular or extracellular way via nucleation (Singh, 2015).
Iron oxides are of nanoparticles that considering the specific inherent characteristics have a wide range of applications in several regions. They can be used as catalysts, energy storage systems, fuel cells and contrast agents in imaging techniques. Magnetite (Fe_3O_4) and hematite

[1]- Hamid Mollania- hamid.mollania69@gmail.com

(Fe_2O_3) are two well-known and important species of iron oxide which have been in the investigators' attention in recent years. A variety of plant extract that are used in Fe_3O_4 nanoparticle synthesis processes have several agents such as phenols and flavenoids and reducing processes are involved in magnetic iron oxide nanoparticles biosynthesis.
In the current study a simple biological and cost-effective method for producing magnetic iron oxide nanoparticles using *crocus sativus L. petal hydro-alcoholic extract*. *Crocus sativus L., (Iridaceae)* commonly known as saffron, that is widely cultivated in Razavi Khorasan Province of (Kumar, V. Z. et al. 2011; Vijayakumar, R., V et al. 2011). Various effective parameters on nanoparticles formation process and probable mechanism of biosynthesis were evaluated respectively. The produced nanoparticles were investigated in terms of chemical composition, size and shape using common analyses.

Materials and Methods

Biosynthesis of magnetic iron oxide nanoparticles using *crocus sativus L.* petal hydro-alcoholic extract: Evaluation of effective parameters on nanoparticles formation and Characterization of synthesized nanoparticles.
In order to biosynthesis MIONPs using plant extract, the plant (*crocus sativus L.* petal) was collected from Torbat-e-Heydarieh, Zaveh. The petals were dried in darkness and low temperature. The dried and fine saffron petals were extracted with a methanol, ethanol, hexan, and water in the ratio of 1:10 w/v. Then it kept in a shaking incubator at ambient temperature for 48 h. The extract were concentrated at 50°C in a rotary evaporator. Various extracts were used for nanoparticles biological production; in a way that the iron salt concentration in the desired volume of extract is considered to be 5 mM. Magnetic iron oxide nanoparticles biosynthesis can be determined at the room temperature (25°C) using $FeCl_3.6H_2O$ after 2 hour and using $FeCl_2.4H_2O$ after 6 hour, as color changes from clear golden yellow to turbid brown. Then the obtained solution was transferred to a special rotary balloon, and it was concentrated as much as possible by rotation at 20-30 rpm, steam bath temperature of 60°C, and circulator temperature of 15-20 °C. The concentrated solution including nanoparticles was poured into a sterile glass plate.
Discovering and understanding factors affecting the nanoparticles formation is amongst the important issues facing the biological production. Therefore, the effect of various factors on biosynthesis of MIONPs in extracellular method was investigated using Ultraviolet and Visible Spectroscopy analysis (UV-Vis) as follows. At first, with the aim to choose suitable precursor salt to be used in the next steps, UV-Vis absorption comparative spectrum for $FeCl_3.6H_2O$, $FeCl_2.4H_2O$ and $FeSO_4.7H_2O$ salts was drawn. In order to investigate the effect of temperature on magnetic iron oxide nanoparticles biosynthesis, nanoparticles synthesis process was performed at different temperatures. Then nanoparticles biosynthesis was investigated by comparing UV-Vis absorption spectra. In the other part, the production of nanoparticles was investigated at pH values of 3.5, 5.5, 7.4, 8.5, and 11 by UV-Vis analysis. Other conditions were imposed quite the same.
In electron microscopes instead of using light beams, the electron bombardment of samples is used for imaging. For field emission scanning electron microscope (FE-SEM) analysis, the

solid powder nanoparticles obtained from concentrating with rotary and after that drying stage was used. High resolution FE-SEM images, indicationg topography and surface characteristics of the nanoparticles and can provide spectrum analysis of elements present in the sample by lateral analysis of EDS. Furthermore, the dynamic light scattering (DLS) is a physical technique to determine the distribution of nanoparticles existing in solutions. This method depends on interaction between light and particle. The scattered light in solution changes as time changes, which can be attributed to the particle diameter. Furthermore, Zeta potential as one of the important factors in defining the amount of nanoparticles electrostatic stability in solution was studied. Both DLS and zeta potential analysis were fulfilled using soluble nanoparticles in supernatant after filtration and at a wavelength of 658 nm.

Results

According to biosynthesis of magnetic iron oxide nanoparticles using ethanolic extarct, the presence of index peak (365 nm) in the range of 350-400 nm is related to iron oxide nanoparticles (Makarov et al., 2014). For factor determination, different salts as precursor of MIONPs were compared. Intensity and position of the peak related to $FeCl_3$. 6H2O is in a more desirable position relative to others. The effect of incubation temperature, ranging from 4-64°C at 20 degrees intervals, on nanoparticles biosynthesis was investigated as a factor with specific effect on the amount of production. The amount of the index peak of 365 nm showed a significant reduction at two threshold temperatures of 4 and 64°C, which can be related to the destruction of possible protein content of extract.

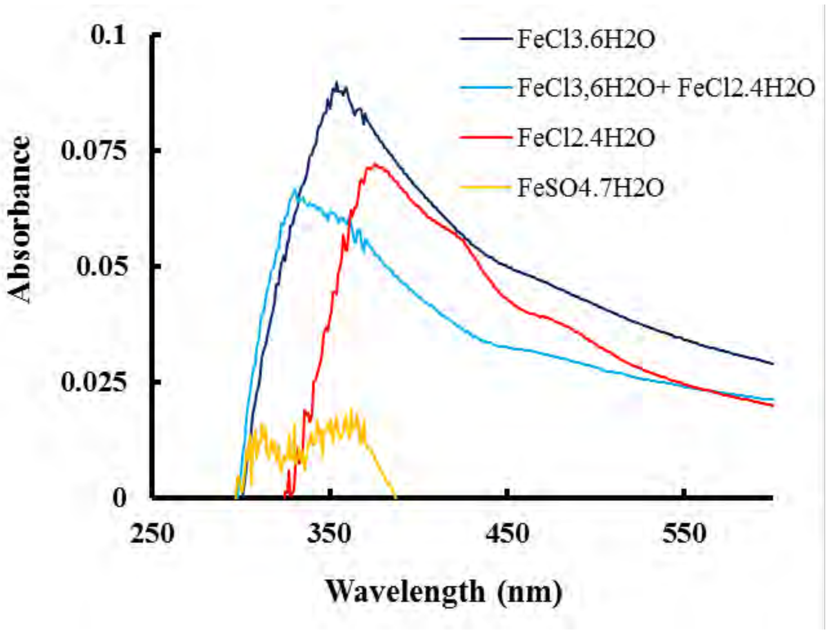
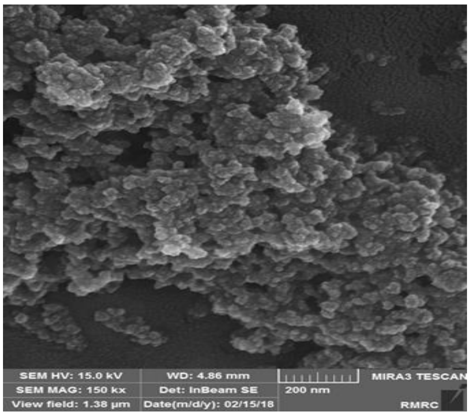


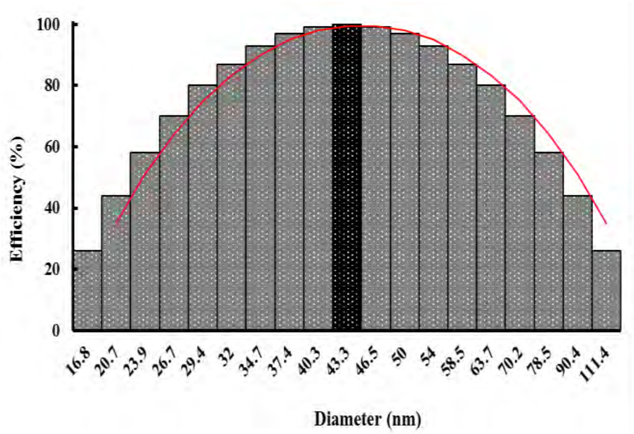
Fig.1: Evaluation of various salt on MIONPs biosynthesis by UV-Vis spectroscopy method

To investigate nanoparticles morphology, crystals shape, their size and placement in the sample, high magnification, and resolution of field-emission scanning electron microscopy (FE-SEM) were studied. The MIONPs, which were produced using ethanolic extract of saffron petals, have spherical and uniform shape.

In order to examine purity and analytical spectrum of nanoparticles in the sample, EDS elemental analysis was done. The peaks associated to Fe are recognizable on both sides of the spectrum. The main compositions are C, N and O that may indicate the presence of proteins and peptides together with nanoparticles. Also Na, Cl and K are attributed to the materials used in magnetic nanoparticles stabilization techniques at the time of analysis. The results found from DLS analysis showed that particles produced in supernatant solution are in the range of 16.8-111.4 nm with the medium size of 43.3 nm. Zeta potential analysis also shows that produced magnetic nanoparticles are approximately uniform in terms of surface charge distribution and indicates that the mean surface charge is equal to -10.6 mV .

B





A

Fig. 2: (A) FE-SEM micrographs of biosynthesized nanoparticles, (B) Size distribution of biosynthesized nanoparticles

Conclusion

The biosynthesis of MIONPs using *crocus sativus L.* petal hydro-alcoholic extract provides a simple, fast, cost-effective and eco-friendly method for synthesizing one of the useful nanomaterials in nanomedicine. Produced nanoparticles have displayed uniform spherical shape with an average size of 18.8-28.3 nm and negative surface charge.

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