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Development of a new method for the determination of glucose in foods using an enzyme-like activity of Fe_3O_4 magnetic nanoparticles

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Abstract
In this study, it was aimed to produce Fe_3O_4 magnetic nanoparticles from the Euphorbia plant (*Euphorbia amygdaloides*) using a simple and economical green biosynthesis method as a new method. Iron oxide nanoparticles have a large number of applications (health, drug development, biosensor, imaging systems etc.) One of these Fe_3O_4 magnetic nanoparticles (MNPs) has been found to be used repeatedly in various application areas due to their properties. Fe_3O_4 MNPs were synthesized using euphorbia (*Euphorbia amygdaloides*) plant extract and $Fe^{+2/+3}$ solution. The characterization analysis was performed using UV-Vis spectrophotometer, SEM, XRD and FTIR devices. Then, the mimetic (enzyme-like activity) properties of MNPs were investigated. It was determined that Fe_3O_4 MNPs mimics enzymes. They have similar properties and catalysis power to natural peroxidases. In the targeted new method, glucose oxidase (GOx) enzyme with Fe_3O_4 MNPs which mimic peroxidase have been used for developing a spectrophotometric method for highly sensitive and stable - enzymatic glucose determination in different foods. The method developed was found to be linear in the 0.25-1 g/L glucose range. The optimal operating parameters of the biosensor were found temperature, (45 ºC), pH (4.0), metal ion concentration (5 mM) and H_2O_2 concentration (12.5 Mm), respectively. In addition, this method has shown a high selectivity to glucose compared to other sugars such as sucrose, lactose, fructose and raffinose.

Keywords: enzyme mimic activity, Fe_3O_4 magnetic nanoparticles (MNPs), glucose, biosensors

1. Introduction

A number of techniques have been developed for easier and more efficient synthesis of nanoparticles. Three basic techniques were used for the synthesis of nanoparticles: physical method, chemical method and biological method. What is important in the synthesis of nanoparticles is the development of reducing agents and stabilizing agents used in the reduction of metal ions. In the method of physical and chemical synthesis, highly toxic and dangerous chemicals are used as reducing agent and stabilizing agent and therefore carry the risk of toxicity to the environment and living things. Therefore, these methods are not useful and applicable for human health (Zhang *et al*. 2016) [16]. However, in the green synthesis method, these agents are naturally present in the biological organisms used, and substances or chemicals having biocompatible biocompatibility that are generally non-toxic and useful as environmental effects have been used. In addition, the most acceptable method is the eco-friendly and non-toxic green synthesis method due to the fact that physical and chemical methods are complex and very expensive and require the use of toxic substances (Hussain, 2016) [5]. (Kumar *et al.* 2015) [6].

Microorganisms (bacteria, yeasts, molds and algae) and plant extracts have been widely used in the synthesis of nanoparticles by green synthesis method (İnci 2017). Nanoparticle synthesis was carried out using the reducing properties of molecules, enzymes, amines, phenolic compounds, alkaloids found in plants and microorganisms. It appears to be the most suitable plant materials for the large-scale biosynthesis of nanoparticles. The nanoparticles are produced by the plants which are more stable and the rate of synthesis is faster than the microorganisms (Padil 2016). Magnetic iron oxide nanoparticles have been used in various fields due to their unique properties such as large surface area and simple separation with magnetic field. The successful use of iron oxide nanoparticles, which are indispensable for a number of fields, in enzyme immobilization, protein purification and food analysis is increasing day by day (Cao *et al.* 2012) [1]. As the most abundant organic compound in the biosphere, carbohydrates play an important role in many of the metabolic systems, their analysis is of great importance in the food, agriculture and health sciences. The selectivity of conventional methods is low and the sensitivity is very low. For chromatographic methods or capillary electrophoresis methods, analytes need to be derivative due to the high number of signal detection or lack of determination limits for some analytes (Özdemir 2010). Natural enzymes have some serious disadvantages such as the ability to easily inhibit catalytic activities and to be digested by proteases. Fe_3O_4 magnetic nanoparticles (MNPs) have similar enzyme-like structures that mimic real enzymes. Generally, magnetic nanoparticles (MNPs) in biology and chemistry are considered to be ineffective (Wei *et al.* 2008). However, in our study, Fe_3O_4 magnetic nanoparticles were preferred because of the mimetic property of peroxidase enzyme. We aimed to determine glucose in different samples by using these properties of metal nanoparticles (Gündüz VD 2017). The determination of glucose in foods and biological fluids has a very

important role in the analysis and control of foods, diagnosis of diseases and determination of the effects of drugs (Galant *et al.* 2015) [2]. We aimed to determine the glucose in different samples (food, blood and serum) by combining the peroxidase-like activities of the magnetic metal nanoparticles and the enzyme glucose oxidase (Gündüz VD 2017).

2. Material and Method

2.1 Green Synthesis of Fe_3O_4 nanoparticles

Synthesis of magnetic iron nanoparticles has been achieved by using 1 mM $FeCl_2$-$FeCl_3$ solution and Euphorbia plant using green synthesis method as Nadaroglu and the group previously did (2017) (Nadaroglu 2017, Karaduman 2017).

2.2 Characterization of Fe_3O_4 nanoparticles

The resulting magnetic iron nanoparticles were identified and characterized using UV-VIS-NIR (Shimadzu UV-3600 Plus), SEM (Scanning Electron Microscope) (Zeiss brand), XRD (Panalytical Empyrean brand) and FT-IR (Fourier Transform Infrared) after the washing and drying steps.

2.3 Investigation of peroxidase-like activity of Fe_3O_4 magnetic nanoparticles

The magnetic iron nanoparticles obtained were measured using the substrate of the enzyme to determine if it had peroxidase activity. For this purpose; 24 µL of the ABTS, 60 mM was mixed with Fe_3O_4 MNPs and 24 µL of the H_2O_2 were added and the enzyme activity was determined by measuring UV-Vis spectrophotometer (417 nm) and calculated. One EU of peroxidase enzyme was defined that oxidize 1.0 µmole of ABTS per minute (Güngör 2011). After the method was optimized by using Nano-Fe_3O_4 peroxidase enzyme, the amount of glucose was decreased according to the following principle in Fig 1.

2.4 Preparation of glucose standard concentrations

To investigate the response of the method at different concentrations of glucose from 0.25 g/L to 1 g/L were analyzed at different concentrations. Using the UV-Vis spectrophotometer, the absorbance values of all samples were measured at a wavelength of 417 nm and the zero adjustment of the spectrophotometer was performed using a blind solution before each measurement.

2.5 Fruit juice, milk and similar samples

To be able to perform glucose analysis, all samples must be clear and homogeneous. For this purpose, - the cloudy structure in the fruit juices was tried to get a clear image by passing them through filter paper. Glucose analysis was carried out after the sample was diluted by 1/2.

2.6 Samples of carbonated beverages and the like

The sample is mixed for 3 minutes at 35 ° C to separate carbonic acid from the sample. Glucose analysis was carried out after the sample was diluted at ½ rate (v/v).

2.8 Jam and similar samples

After the sample is homogenized, 5 g sample was completed to 20 mL with pure water. Then, the solution was homogenized with a heater and then, it was passed through a filter paper. Glucose analysis was carried out by diluting the sample to ¼ (v/v).

2.9 Honey samples

After stirring the honey, 20 gr was taken with spatula and weighed on the sensitive balance. After being homogenized by stirring with a heater at 60 ° C for 15 minutes, it was waited to cool down. 5 g of honey sample was weighed and dissolved in a total of 20 mL of distilled water. Peroxide analysis was carried out by diluting the sample to ¼ (v/v).

In order to compare the method, a similar spectroscopic and colorimetric based kit was purchased. The working principle of the kit was similar to ours but based on the use of different substrates and two separate enzymes. Glucose analysis kit and also the results of the studies were carried out by HPLC method are examined and the results are compared.

3. Results & Discussion

3.1 Determination of Glucose by Using Fe_3O_4 Magnetic Nanoparticles

In order to investigate the response of the method at different concentrations of glucose from 0.25 g/L to 1 g/L were analyzed at different concentrations. The color changes in the result of analysis at different concentrations were shown in Fig. 2.

In Fig. 3, linearity was observed when the concentration graph was drawn against absorbance in the range of 0.25-1 g/L glucose concentration. Each error bar 5 showed the standard deviation for the measurement.

The concentration absorbance graph was drawn by using glucose standard solutions which was prepared at different concentrations and the glucose concentrations of the samples were calculated using the observed equation from this graph (1).

$$\text{Glucose Concentration (g/L)} = \frac{\text{Absorbance Increase Measured for Sample}}{\text{The slope of the calibration graph}} \quad (1)$$

After optimizing the method we developed by using Fe_3O_4 magnetic nanoparticles, the glucose analysis was performed using the glucose analysis kit method to compare the accuracy of the measurement results. In the glucose assay kit, glucose oxidase and peroxidase enzymes as well as a different colorimetric substrates can be used to perform glucose analysis. The comparison of the commercially available glucose analysis kit and the Fe_3O_4 MNPs based method assay method was done by measuring the amounts of standard glucose samples in different concentrates and the results were given in Fig. 4.

The selectivity of the method based on Fe_3O_4 MNPs was performed for glucose in the concentration range of 0.25 (g/L) - 1 (g/L), as shown in Figs. 3 and 4. Compared to the other method, the data obtained from Fe_3O_4 MNPs were found to be closer to the standard for all glucose concentrations. The Fe_3O_4 MNPs synthesized by the green synthesis method and the new determination method using the GOx enzyme were tested for specificity by using different sugar samples to test the specificity of glucose determination and the results of the new method were measured. The measurement results of 5 mM sucrose, lactose, fructose, raffinose and glucose amounts in solutions under the same conditions are shown in Fig. 5. As it can be seen from the figure, it was observed that other sugars did not adversely affect the amount of glucose determination.

Analysis of glucose content in food samples according to different methods and newly developed method was used for comparison. Glucose amount was determined for some food samples such as apricot juice, peach juice, molasses, honey, milk, cola, strawberry jam and apricot jam using the new method. Glucose determination assays were repeated using the glucose analysis kit for comparing to the new method results. In the same operating conditions, biosensor system response was measured by two different methods. The results are given in Table 1 in comparison with the glucose analysis kit method. In order to be a reference, the amount of glucose in the foods determined from the TURKOMP database was also used (TURKOMP 2018).

As it can be seen in Table 1, it was determined that the results obtained by using Fe_3O_4 magnetic nanoparticle and GOx in foods were more compatible with the database (TURKOMP 2018).

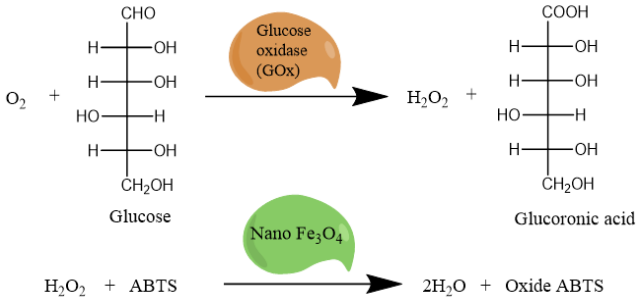

Fig 1: Analysis principle

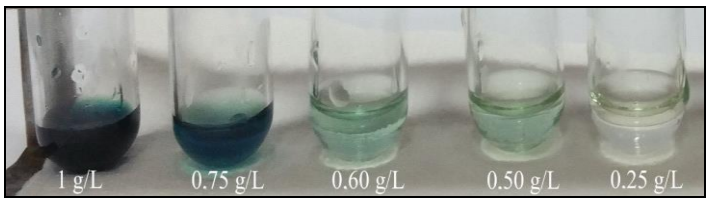

Fig 2: Color change observed during glucose determination with different glucose concentrations

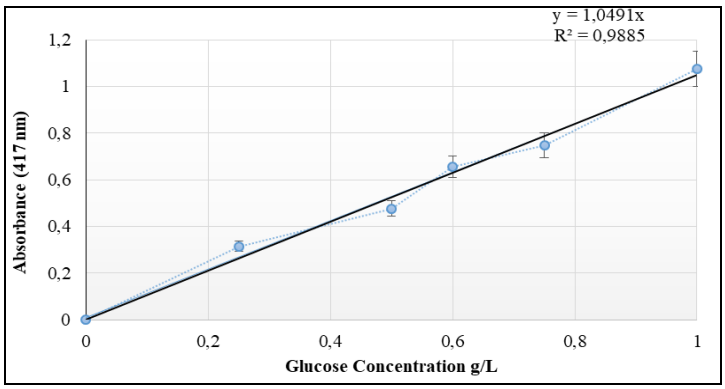

Fig 3: Fe_3O_4 magnetic nanoparticle based glucose determination method calibration chart

(20 µL GOx, 200 µL (0,25 g/L, 0.50 g/L, 0.60 g/L, 0.75 g/L and 1 g/L), 24 µL60 mM ABTS, 10 µL Fe_3O_4, 800 µL 0,2 mM acetate buffer

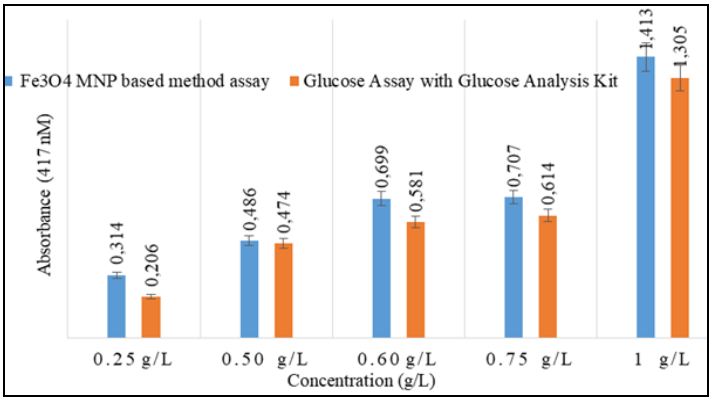

Fig 4: Comparative glucose determination results of glucose kit based determination method by new method of Fe_3O_4 MNPs

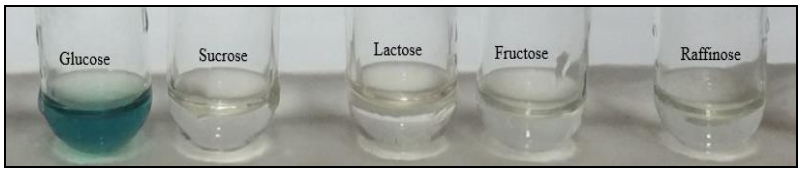

Fig 5: Color changes observed in the glucose determination method with colorimetric method developed by using Fe_3O_4 MNPs

Table 1: The amount of glucose in some foods.

Food sample	Fe_3O_4 MNPs Based Spectrophotometric Method (gr/L)	Glucose Analysis Kit with Spectrophotometric Method (gr/L)	The Amount of Glucose in Foods from the Literature HPLC (gr/L)
Apricot juice	61.0 ± 2.4	39.6 ± 1.9	68.2
Peach juice	51.1 ± 2.0	39.7 ± 1.9	56.1
Boiled grape juice	232.9± 9.3	112.9 ± 5,6	240.4
Honey	246.7 ± 9.8	134.5 ± 6.7	283.9
Milk	40.4 ± 1.6	26.5 ± 1.3	50.0
Cola	46.8 ± 1.8	39.2 ± 1.9	52.3
Strawberry jam	201.1± 8.0	157.3 ± 7.8	215.5
Apricot jam	150.1 ± 6.0	113.4 ± 5.6	149.0

5. Conclusions

Thanks to the surface bio-conjugation with molecular probes, excellent biocompatibility and less toxicity properties, green biosynthesis can be controlled using shape, size and inequality, great physical and chemical inertness, optical properties associated with surface plasmon resonance, surface modification, cost-effective, environment-friendly preparations Green synthesis also minimizes the need to use hazardous chemicals and reduces the cost of synthesis with the use of waste materials (Hussain, *al.* 2016) [5]. In this study made by our group, the properties of this base of green synthesis were utilized. The effect of pH and temperature on wavelength, Fe_3O_4 Magnetic nanoparticles and H_2O_2 (Hydrogen peroxide) were investigated. As a result; the glucose biosensor we prepared in this study, the linear working range (0.25-1 g/L) was determined and glucose was determined in this range. It has been determined that it has the same linear working range as the commercially available glucose analysis kit. As a result of the experimental investigations, the optimal operating parameters of the biosensor was temperature (45 ºC), pH (4), concentration of metal ion (5 mM) and H_2O_2 concentration (12.5 mM). Amount of some other sugars such as sucrose, lactose, fructose and raffinose using the developed method. More importantly, a sensitive and selective method for glucose detection was developed using glucose oxidase (GOx) and prepared Fe_3O_4 MNPs. The detection platforms for glucose developed in this study not only confirmed that Fe_3O_4 MNPs had biomimetic peroxidase-like activity, but also showed great potential applications in the future of a variety of analytical approaches which should be simple, robust and easy to do. As a result; the experiments performed on different samples were performed and succeeded with Fe_3O_4 MNPs which mimic the peroxidases obtained by green synthesis method. A new biosensor to be developed by this method has been determined as a result of our investigations that it can be used in food, clinical and many other fields. The method developed showed a satisfactory performance with sensitivity, selectivity and long-term stability. Moreover, it is thought that the analyte used will be preferred for environmental friendly and economic reasons that MNPs can be used repeatedly because of its magnetic and non-degradable properties.

6. References

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