Spectrophotometric determination of the Levofloxacin using green nanochemistry applications

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Abstract
In this study, a simple, rapid and accurate spectrophotometric method was proposed for the determination of the Levofloxacin using Nickel Oxide nanoparticles NiONPs (which were synthesized in a green way using dried mint leaves extract), It gave the highest value of absorption at 294 nm wavelength, and the linear relationship followed the Beer-Lambert law in the range of concentrations (2-24 µg/mL) with a good correlation coefficient, where its value was $R = 0.9996$ which indicates good linearity, the value of the molar absorptivity $\varepsilon$=2.9704×10^{4} L/mol.cm , and the value of the Sandell's significance $S$= 0.0122 µg/cm² . The validity of the proposed method was validated statistically and quantitatively, as the detection limit value $L_{d}$ = 0.2799 µg/mL and the quantitative limit value $L_{q}$ =0.9330 µg/mL. The method proved to be of good accuracy and precision, as the recovery rate was $\%\text{Rec} =98.05$, and the relative standard deviation $\%\text{RSD}$ did not exceed 0.42 . The formation of these particles was tested using FT-IR technology and scanning electron microscopy (SEM), and the binding of the drug to the nanoparticles was confirmed by using UV-VIS spectroscopy.

Keywords:
Green chemistry, Levofloxacin, Nanoparticles, Spectrophotometric, Diagnosis.

Introduction:

Green chemistry: It is one of the modern branches that have been directed to, and concerned with preparing materials that are not harmful to the environment and therefore it is considered a typical way to reduce pollution in the environment\textsuperscript{(1)}, there are several developments that have taken place to expand the work of green chemistry and make it apply to organic and inorganic chemistry, physical chemistry, biochemistry and analytical chemistry in order to reduce damage in these areas as well as reduce the use of substances harmful to humans and the environment\textsuperscript{(2)}.

Green nanotechnology: Green nanotechnology is considered one of the branches of green technology that uses the concepts of green chemistry and green engineering. Nanotechnology products and processes have contributed to protecting the environment and climate by providing raw materials, energy and water, as well as by reducing greenhouse gases and harmful waste by converting them into nanomaterials. It ranges from (1-100)
nanometers\(^{(3)}\). It also contributed to changing the medical rules used in preventing, diagnosing and treating diseases by introducing new methods for nano drug carriers inside the body that are able to target different cells in the body\(^{(4)}\), and that the role of Phytochemicals in general synthesis, architecture and other aspects involving nanoparticles are very attractive because they produce a much-needed equivalence between plant science and nanotechnology, and this equivalence provides development in the field of nanotechnology\(^{(5)}\), and the main motivation for the use of plant extract in the production of nanomaterials It is easily available and safe and does not cause any toxic damage, as in the chemical preparation, which leads to the formation of a toxic chemical substance absorbed on the surface, which has a negative effect when used in the medical field\(^{(6)}\), and that the main reaction that occurs when using plant extract is the oxidation/reduction reaction\(^{(7)}\).

Nanotechnology has several advantages, including the ability to control single atoms accurately and rearrange them, and the adoption of nanotechnology on the principles of chemistry, biology, physics, electronic and electrical engineering, and thus it brings together different fields of science and contributes to the link between these fields, as well as its reliance on scientific research that can be applied in order to enable us to transform imagination Scientific into reality, and the possibility of controlling atoms where it is possible to manufacture materials and devices and rid them of defects to make their properties better in all respects, as well as the physical and chemical properties of the nano-sized material differ from the properties of the material itself at its natural size and thus provided us with the possibility of identifying new and advanced properties that can be used in many fields of applied sciences\(^{(8)}\).

Nanomaterials: The dimensions of nanomaterials range from (1-100) nanometers, and the smallness of these materials led to different properties from materials in their normal scale (greater than 100 nanometers)\(^{(4,9)}\), and nanoparticles have more functional capabilities than microparticles because of it has more surface atoms, and this distinction made it enter in many fields such as medicine, stimulation, sensors, optoelectronics, and in the field of agriculture, textile industry, and food industries\(^{(10)}\).

Nickel nanoparticles: Nickel nanoparticles have magnetic properties, and this is what made them receive a lot of attention because of their effective applications in the field of magnetic storage, magnetic cooling, and other applications that depend on the magnetic characteristic \(^{(11)}\).

Nickel nanoparticles are prepared by several methods such as photoreduction\(^{(12)}\), radioreduction\(^{(13)}\), sonochemical method\(^{(14)}\), solvent extraction reduction\(^{(15)}\), micro-emulsification\(^{(16)}\) and alcohol reduction\(^{(17)}\).

Nickel nanoparticles have applications in many fields including electronics, magnetism\(^{(18)}\), energy technology\(^{(19)}\), and biomedicine\(^{(20)}\) due to their high reactivity, operational simplicity, and environmentally friendly properties. One recent application is their role in manufacturing of carbon nanotubes\(^{(21)}\), as well. It has useful environmental applications such as the adsorption of hazardous dyes and inorganic pollutants and thus has a major role in environmental cleanliness\(^{(22)}\).
Levofloxacin: which is (23) (s) 9-fluoro-2, 3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2, 3-de]-1,4-benzoxazine-6-carboxylic acid, hemihydrates, and its structural formula is as in Figure (1):

![Structural formula of levofloxacin](image)

**Fig. 1:** The structural formula of levofloxacin

Its molecular formula is C_{18}H_{20}FN_{3}O_{4} and its molecular weight is 361.368 g / mol. It is a white to yellowish powder (24), it has poor solubility in water or methanol, it dissolves in glacial acetic acid, its melting point is 218 °C and its boiling point is 572 °C (25). Levofloxacin is considered A new antibiotic from fluoroquinolones (26), which is the active isomer of fluoroquinolones, and the activity of levofloxacin is 8,128 times higher than the activity of ofloxacin, and this means that bacterial species that are resistant to ofloxacin and the first generation of fluoroquinolones (for example, pneumococci and organisms that cause atypical pneumonia) are more Sensitivity to levofloxacin (27), and its ability to confront bacteria lies in preventing the reproduction of bacterial DNA (28,29), and it has a broad spectrum against gram-negative and gram-positive bacteria, as it is used to treat many infections caused by these bacteria such as bronchitis, sinustis, pneumonia, urinary tract infection, dermatitis, pelvic nephritis, plague and anthrax(30).

**Materials and method:**

In this study, dried mint leaves were used, which were provided by nature, after collecting mint leaves, washing them well, drying them in the air, then exposing them to the sun for a week, and then grinding them. Aqueous nickel chloride, NiCl_{2}.6H_{2}O, was used, which was provided by Fluka, by taking 2.3760 g of it and dissolving it in a little deionized water, then completing the volume (to the mark) in a volumetric flask of 100 mL to obtain a solution. At a concentration of 0.1 M.

10 g of dried mint leaves were taken and added to 100 mL of deionized water, then boiled for 15 minutes, left to cool, then filtered with filter paper to obtain an extract from which 50 mL was taken and added to 100 mL of (0.1 M) NiCl_{2}.6H_{2}O solution and placed on Magnetic stirring for four hours while adjusting the pH of the mixture up to 8, using sodium hydroxide solution (0.1 M). At the end of the magnetic stirring process, the colour of the mixture changed to brown, and a precipitate formed at the bottom. The mixture was filtered with filter paper and the precipitate was dried for 6 hours in a drying oven at 100°C, then the precipitate was placed in a calcination oven at 350°C for four hours. The material formed after the calcination process was collected and 0.1 g of it was taken and placed in a container with a little of deionized water in the ultrasonic water bath device for 15 minutes at 60 °C, then the
volume was completed (up to the mark) in a volumetric flask of 100 mL in order to obtain a NiONPs solution for use in experiments later (31,32).

The main idea of the study is to show the effect of wavelength when adding levofloxacin to nanoparticles, where when measuring the wavelength of levofloxacin in its pure form, its value was 289 nanometers, and the principle of the method depends on adding 1 ml of levofloxacin (at a concentration of 100 μg / ml) to 5 mL of NiONPs solution, then dilute it with deionized water in a 10-mL volumetric flask (to the mark) and measure the absorbance spectrum of the resulting solution. It was found that the largest value of absorption was at 294 nm.

The reaction conditions were adjusted by conducting several spectroscopic measurements to show the effect of several factors, including the effect of hydrochloric acid, the best possible volume of acid to be added to the solution, effect of temperature, as well as the best volume of the nanomaterial solution. These measurements were made using a volumetric flask with a capacity of 10 mL. The results were as follows:

The effect of hydrochloric acid was clear as it led to an increase in absorbance, as shown in Figure (2):

![Fig. 2: UV-VIS for (A) Levo +NiONPs+ HCl, (B) Levo+ NiONPs](image)

The best volume of hydrochloric acid that was added to the mixture of the nano-solution and the drug (1 mL of Levo and 5 mL of NiONPs) is 1.7 mL, as shown in Figure (3):
**Fig. 3:** The best volume of HCl

The best volume of NiONPs solution is 3.6 mL, as shown in Figure (4):

**Fig. 4:** The best volume of NiONPs

The temperature had a clear effect on the absorbency of the solution, where the final solution was stable in the range 25-30 degrees of temperature, as shown in Figure(5):

**Fig. 5:** The effect of temperature
The stability of the final product was studied using the ideal volumes and conditions that were followed in the method. It was found that the product remained stable during the first 20 minutes of its preparation and gave the highest absorption of the solution. As shown in Figure (6):

After adjusting these conditions, a calibration curve for levofloxacin was prepared by taking increasing concentrations of the drug from 2-24 μg / mL and adding to 3.6 mL of NiONPs solution and 1.7 mL of HCl at pH = 1.2 and dilution with deionized water in a volumetric flask It has a capacity of 10 mL and measured the absorbance of the solutions at the wavelength of 294 nm. The result of the calibration curve was as shown in Figure (7). The value of the molar absorptivity was $2.9705 \times 10^4$ L/mol.cm, the Sandell’s significance value was 0.0121 µg/cm², and the correlation coefficient $R$ was 0.9996.

Accuracy and Precision

This test was carried out by taking three values of concentrations (within the limits of Beer-Lambert law located within the calibration curve) of the levofloxacin solution shown in the table, at an average of (three readings) for each concentration and from the results of calculating the "recovery rate" (Rec.%) and the "standard deviation" Relative" (RSD%) It was found that the used method has high accuracy and good compatibility, as shown in Table (1):
### Table 1: Accuracy and Precision

<table>
<thead>
<tr>
<th>Taken Levo µg/mL</th>
<th>Abs.</th>
<th>Found Levo µg/mL</th>
<th>%Recovery</th>
<th>Av. Of % Recovery</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.419</td>
<td>3.83</td>
<td>95.75</td>
<td>98.05</td>
<td>0.20</td>
</tr>
<tr>
<td>10</td>
<td>0.921</td>
<td>9.94</td>
<td>99.40</td>
<td>99.40</td>
<td>0.18</td>
</tr>
<tr>
<td>20</td>
<td>1.732</td>
<td>19.80</td>
<td>99</td>
<td>99.05</td>
<td>0.42</td>
</tr>
</tbody>
</table>

### Limit of Detection and Limit of Quantitation

The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated based on the following mathematical relationship:

\[
\text{LOD} = 3\delta / S \\
\text{LOQ} = 10\delta / S
\]

Where \(\delta\) = 0.0076 it means the relative deviation of the intersection resulting from the straight line equation \(Y = aX + b\), i.e. the value of \(b\), and it is calculated by repeating the calibration curve five times.

\(S\) means the value of the slope of the curve, the value of the detection limit was 0.2799 µg/mL and the quantitative limit was 0.9331 µg/mL.

### Applying the method to the pharmaceutical preparation

The direct method was applied to the Livofloxacin pharmaceutical preparation (Uniflox 500 mg), where three different concentrations of the preparation solution were taken, which are 4, 6, 10 µg/mL, the solution was treated with the same steps for preparing a calibration curve for a pure solution of Levofloxacin, and measuring its absorption at a wavelength of 294 nm, as well as calculating the recovery rate and its relative standard deviation, as in Table (2)

### Table 2: The results of application direct method for determination Levo in pharmaceutical preparation

<table>
<thead>
<tr>
<th>Taken Levo µg/mL</th>
<th>Found Levo µg/mL</th>
<th>%Recovery</th>
<th>Av. of % Recovery</th>
<th>%RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>3.83</td>
<td>95.75</td>
<td>98.05</td>
<td>0.19</td>
</tr>
<tr>
<td>6</td>
<td>5.77</td>
<td>96.18</td>
<td>97.12</td>
<td>0.31</td>
</tr>
<tr>
<td>10</td>
<td>9.94</td>
<td>99.44</td>
<td>99.05</td>
<td>0.07</td>
</tr>
</tbody>
</table>

*Average of five readings

### Diagnosis of nanoparticles

**UV-VIS:** Ultraviolet-visible spectroscopy was used to know the association of levofloxacin with nickel nanoparticles, where a displacement occurred in the wavelength value from 260 nm to 294 nm, and this shift in wavelength indicates the change in the surface chemistry of NiONPs as a result of the association of levofloxacin surface of nickel oxide nanoparticles, as shows in Figure (8):
FT-IR: FT-IR spectroscopy was used in this study to detect the formation of NiONPs. This technique indicates the presence of several active groups, which were questioned by the appearance of several packages, where the two bands at (461 and 443.64 cm\(^{-1}\)) refer to the Ni-O bond, and a band at 1037.74 cm\(^{-1}\) belongs to the C-O bond, and the band at 1627.97 cm\(^{-1}\) belongs to the C=C bond, and the band at 2926 cm\(^{-1}\) belongs to the C-H bond, and bands at 3404.47 cm\(^{-1}\) and 3421.83 cm\(^{-1}\) belong to the bond O-H as shown in Figure (9):

**Fig. 9:** FT-IR spectroscopy of NiONPs

SEM: The imaging SEM technique was used to clarify the shapes of the formed nanoparticles, as they are produced in the form of triangular images Dimensions, scanning electron microscopy (SEM) imaging of the prepared nickel oxide nanoparticles showed agglomerates. The nanoparticles have almost regular spherical shapes and are scattered randomly, as shown in Figure (10), and the sizes of these granules range between (15.4 - 53.8 nm), and the imaging showed a lot of gaps (dark areas). This shows the high porosity of the sample and therefore is expected to be good in adsorption applications and gas storage.
Fig. 10: A&B The imaging SEM for NiONPs

Conclusion:

It was concluded from this study the success of the method used in the production of nanomaterials from green sources, and thus the trend towards green manufacturing methods became more desirable because it is characterized by ease of preparation, low cost and no need to use materials harmful to the environment, and it was also concluded that when the drug levofloxacin was associated with NiONPs increased The wavelength value ranged from 260 nm (for NiONPs) to 294 nm when adding the drug to the nanoparticle solution (taking into account ideal conditions) as evidence of the association between these particles and the drug solution, and the final solution followed Beer Lambert's law in the range of concentrations 2-24 µg/mL of levofloxacin solution, the value of the molar absorptivity was $2.9705 \times 10^4$ L/mol.cm, the Sandell's significance value was 0.0122 µg/cm², and the correlation coefficient $R$ was 0.9996, when calculating the %Rec. It was equal to 98.05 and the RSD% value did not exceed 0.42.

References

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التقدير الطيفي لليفوفلوكساسين باستخدام تطبيقات الكيمياء الخضراء النانوية

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الخلاصة:
في هذه الدراسة، تم اقتراح طريقة طيفية بسيطة وسريعة ودقيقة لتقدير الليفوفلوكساسين باستخدام جسيمات أوكسيد النحاس النانوية (NiONPs) التي تم تصنيعها بطريقة خضراء باستخدام مستخلص أوراق النعناع المجففة، حيث أعطي المحلول الناتج أعظم قيمة للإمتصاص عند الطول الموجي 294 النانومتر، وكانت العلاقة الخطية تلت قانون بير-لامبرت في مدى التراكيز 2-24 مايكروغرام/مل، وبلغت قيمة معامل الارتباط 0.9996 مما أكّد الخطية الجيدة، وكانت قيمة معامل الامتصاص المولاري = 4.29704×10^{-4} لتر/مоля/سم، قيمة دلالة ساندل = 0.0122 مايكروغرام/سم². تم التحقق من صحة الدراسة الطيفية المترادفة إحصائياً وكمياً، حيث بلغت قيمة حد الكشف = 0.2799 مايكروغرام/مل وقيمة الحد الكمي = 0.9330 مايكروغرام/مل. أثبتت الطريقة أنها ذات دقة وتوافقية جيدة، حيث كان معدل الاسترجاع 98.05 %، ولم يتجاوز الانحراف القياسي % RSD عن 0.42 %، تم اختبار تكوّن هذه الجسيمات باستخدام تقنيات FT-IR والمجهر الإلكتروني الماسي (SEM)، وتم تأكيد ارتباط الدواء بالجسيمات النانوية باستخدام التحليل الطيفي للأشعة فوق البنفسجية المرئية UV-VIS.

معلومات المؤلف:
الايميل: 
الموبايل: 

المعلومات البحثية:
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ملاحظات: 
التأريخ الاستلام: 
التاريخ المتغير: 
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الكلمات المفتاحية: 
العنوان:
الايميل: 
الموبايل: